



US011351244B2

(12) **United States Patent**
Calvert et al.

(10) **Patent No.: US 11,351,244 B2**
(45) **Date of Patent: Jun. 7, 2022**

(54) **EFFECTIVE VACCINATION AGAINST
PORCINE REPRODUCTIVE AND
RESPIRATORY SYNDROME (PRRS) VIRUS
PRIOR TO WEANING**

(71) Applicant: **Zoetis Services LLC**, Parsippany, NJ
(US)

(72) Inventors: **Jay Gregory Calvert**, Otsego, MI
(US); **Robert G. Ankenbauer**, Portage,
MI (US); **Jacqueline Gayle Marx**,
Portage, MI (US); **Douglas S. Pearce**,
Kalamazoo, MI (US); **Marcia L. Keith**,
Plainwell, MI (US)

(73) Assignee: **Zoetis Services LLC**, Parsippany, NJ
(US)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(21) Appl. No.: **16/822,830**

(22) Filed: **Mar. 18, 2020**

(65) **Prior Publication Data**

US 2020/0215181 A1 Jul. 9, 2020

Related U.S. Application Data

(63) Continuation of application No. 13/895,564, filed on
May 16, 2013, now Pat. No. 10,610,581.

(60) Provisional application No. 61/648,461, filed on May
17, 2012.

(51) **Int. Cl.**
A61K 39/12 (2006.01)
C12N 7/00 (2006.01)
A61K 39/00 (2006.01)

(52) **U.S. Cl.**
CPC **A61K 39/12** (2013.01); **C12N 7/00**
(2013.01); **A61K 2039/5254** (2013.01); **A61K**
2039/53 (2013.01); **A61K 2039/55** (2013.01);
A61K 2039/552 (2013.01); **C12N 2770/10034**
(2013.01); **C12N 2770/10064** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,137,631 A	6/1964	Soloway
3,519,710 A	7/1970	Bass
3,959,457 A	5/1976	Speaker et al.
4,016,100 A	4/1977	Suzuki et al.
4,205,060 A	5/1980	Monsimer et al.
4,452,747 A	6/1984	Gersonde et al.
4,606,940 A	8/1986	Frank et al.
4,744,933 A	5/1988	Rha et al.
4,921,706 A	5/1990	Roberts et al.
4,927,637 A	5/1990	Morano et al.
4,944,948 A	7/1990	Uster et al.
5,008,050 A	4/1991	Cullis et al.

5,009,956 A	4/1991	Baumann
5,132,117 A	7/1992	Speaker et al.
5,789,543 A	8/1998	Ingham et al.
5,866,401 A	2/1999	Hesse
5,951,977 A	9/1999	Nisbet et al.
5,998,601 A	12/1999	Murtaugh et al.
6,015,686 A	1/2000	Dubensky, Jr. et al.
6,033,886 A	3/2000	Conzelmann
6,042,830 A	3/2000	Chladel et al.
6,268,199 B1	7/2001	Meulenberg et al.
6,500,662 B1	12/2002	Calvert et al.
6,943,152 B1	9/2005	Audonnet et al.
7,132,106 B2	11/2006	Calvert et al.
7,232,680 B2	6/2007	Calvert et al.
7,473,428 B2	1/2009	Johnson et al.
7,618,797 B2	11/2009	Calvert et al.
7,691,389 B2	4/2010	Calvert et al.
7,754,464 B2	7/2010	Calvert et al.
8,058,050 B2	11/2011	Calvert et al.
8,110,390 B2	2/2012	Faaberg et al.
8,420,373 B2	4/2013	Delputte et al.
8,481,705 B2	7/2013	Calvert et al.
8,492,132 B2	7/2013	Calvert et al.
8,609,827 B2	12/2013	Calvert et al.
9,102,912 B2	8/2015	Calvert et al.
9,566,324 B2	2/2017	Calvert et al.
2004/0087521 A1	5/2004	Donnelly et al.
2011/0177118 A1	7/2011	Zuckermann
2012/0213810 A1	8/2012	Burgard

(Continued)

FOREIGN PATENT DOCUMENTS

EP	0839912 A1	5/1998
JP	9313188	12/1997

(Continued)

OTHER PUBLICATIONS

Alignment of SEQ ID No. 6 with U.S. Pat. No. 6,500,662—SEQ ID
Dec. 1, 1999.*

U.S. Appl. No. 60/694,021, Han et al., filed Jun. 24, 2005
Ishizeki, S. et al, Jul. 18-21, 2010, "Evaluation of the efficacy of
PRRSV modified live vaccine in one-day old piglets," Poster
Presentations, p. 232, Proceedings of the 21st IPVS Congress,
Vancouver, Canada.

Markowska-Daniel, I. et al., 2011, "The influence of age and
maternal antibodies on the postvaccinal response against swine
influenza viruses in pigs," Veterinary Immunology and Immunopathol-
ogy, 142:81-86.

(Continued)

Primary Examiner — Shanon A. Foley

(74) *Attorney, Agent, or Firm* — Vyacheslav Vasilyev

(57) **ABSTRACT**

The invention provides isolated polynucleotide molecules
that comprise a DNA sequence encoding an infectious RNA
sequence encoding a genetically-modified North American
PRRS virus, methods to make it and related polypeptides,
polynucleotides, and various components. Vaccines com-
prising the genetically modified virus and polynucleotides
and a diagnostic kit to distinguish between naturally infected
and vaccinated animals are also provided.

5 Claims, 8 Drawing Sheets

Specification includes a Sequence Listing.

(56)

References Cited**U.S. PATENT DOCUMENTS**

2013/0309263 A1 11/2013 Calvert et al.
 2014/0186395 A1 7/2014 Delputte et al.
 2017/0136117 A1 5/2017 Calvert et al.
 2020/0197507 A1* 6/2020 Calvert A61K 39/12

FOREIGN PATENT DOCUMENTS

WO WO 92/21375 12/1992
 WO WO 93/03760 3/1993
 WO WO 96/04010 A1 2/1996
 WO WO 96/06619 3/1996
 WO WO 95/28227 10/1996
 WO WO 96/40932 12/1996
 WO WO 98/55626 12/1998
 WO WO 2007/002321 A2 1/2007
 WO WO 2012/063212 A1 5/2012

OTHER PUBLICATIONS

- O'Neill, K. et al., 2012, "Use of commercial subunit and chimeric vaccines in 5-day-old piglets is effective in protecting from PCV2 viremia and PCVAD in a PCV2, PPV and PRRSV triple challenge model," American Association of Swine Veterinarians Annual Meeting, p. 43.
- Rossow, K. D., 1998, "Porcine reproductive and respiratory syndrome," *Veterinary Pathology*, 35: 1-20.
- Butler, J. E., D. H. Francis, J. Freeling, P. Weber and A. M. Krieg (2005). "Antibody repertoire development in fetal and neonatal piglets. IX. Three pathogen-associated molecular patterns act synergistically to allow germfree piglets to respond to type 2 thymus-independent and thymus-dependent antigens." *Journal of Immunology* (Baltimore, Md.: 1950) 175: 6772-6785.
- Butler, J. E. and M. E. Kehrle (2005). Immunoglobulins and immunocytes in the mammary gland and its secretions. *Mucosal Immunology*. J. Mestecky, M. E. Lamm, W. Strober et al., Academic Press. 2: 1763-1793.
- Butler, J. E., M. Sinkora, N. Wertz, W. Holtmeier and C. D. Lemke (2006). "Development of the neonatal B and T cell repertoire in swine: implications for comparative and veterinary immunology." *Veterinary Research* 37: 417-441.
- Hammerberg, C., G. G. Schurig and D. L. Ochs (1989). "Immuno-deficiency in young pigs." *American journal of veterinary research* (USA)(6): 868.
- Hurley, D. J. (2004). Neonatal immune development in swine management. American Association of Swine Veterinarians, Des Moines, Iowa.
- Kumar, A., A. N. Vlasova, Z. Liu, K. S. Chattha, S. Kandasamy, M. Esseili, X. L. Zhang, G. Rajashekara and L. J. Saif (2014). "In vivo gut transcriptome responses to *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* in neonatal gnotobiotic piglets." *Gut Microbes*, 5: 152-164.
- Lemke, C. D., J. S. Haynes, R. Spaete, D. Adolphson, A. Vorwald, K. Lager and J. E. Butler (2004). "Lymphoid hyperplasia resulting in immune dysregulation is caused by porcine reproductive and respiratory syndrome virus infection in neonatal pigs." *Journal of Immunology* (Baltimore, Md.: 1950) 172: 1916-1925.
- Roth, J. A. (1999). The immune system. *Diseases of Swine*. B. E. Straw, S. D'Allaire, W. L. Mengeling and D. J. Taylor. Ames, Iowa, Iowa State Press: 799-820.
- Sun, X. Z., N. Wertz, K. L. Lager, G. Tobin and J. E. Butler (2012). "Antibody repertoire development in fetal and neonatal piglets. XXIII: fetal piglets infected with a vaccine strain of PRRS virus display the same immune dysregulation seen in isolator piglets." *Vaccine*, 30: 3646-3652.
- Zeidler, R. B. and H. D. Kim (1985). "Phagocytosis, chemiluminescence, and cell volume of alveolar macrophages from neonatal and adult pigs." *Journal of Leukocyte Biology*, 37: 29-43.
- Ausubel, et al., "Current Protocols in Molecular Biology," Greene Publishing Associates & Wiley Interscience, NY (1989).
- Allende, R. et al., EMBL online database, AF046869 PRRS virus 16244B, complete genome Oct. 21, 1998.
- Chasin, M., et al., "Biodegradable Polymers as Drug Delivery Systems," *Drugs and the Pharmaceutical Sciences*, vol. 45 (1990).
- Coligan, J.E., et al, *Current Protocols in Immunology* John Wiley & Sons, Inc. (1998).
- Collins, J.E., et al., "Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR.23 2) in North America and experimental reproduction of the disease in gnotobiotic pigs," *J. Vet. Diagn. Invest.*, 4:117-126 (1992).
- Den Boon JA, et al., *J. Virol.*, 65(6):2910-2920 (1991).
- Domb, A., et al., "Polymers for Advanced Technologies," 3:279-292(1992).
- Erlich, "PCR Technology," Academic Press, Inc. 1992.
- Enjuanes et al., *Journal of Biotechnology* 88:183-203, 2001.
- Innis, et al., "PCR Strategies", Academic Press, Inc. (1995).
- Kim, H.S., et al., "Enhanced replication of porcine reproductive and respiratory syndrome (PRRS) virus in a homogeneous subpopulation of MA-104 cell line," *Arch Virol.*, 133:477-483 (1993).
- Kwang, J., et al., "Cloning, expression, and sequence analysis of the ORF4 gene of the porcine reproductive and respiratory syndrome virus MN-1b" *Journal Bet. Diagn. Invest.* 6:293-296_(1994).
- Kreutz, L.C. "Cellular membrane factors are the major determinants of porcine reproductive and respiratory syndrome virus tropism," *Virus Research* 53:121-128, 1998.
- Mardassi, H., 1995., *Arch. Virol.* 140: 1405-1418.
- Meulenber, J.J.M. et al., "Lelystad Virus, the Causative Agent of Porcine Epidemic Abortion and Respiratory Syndrome (PEARS), is Related to LdV and EAV," *Virology*, 192:62-72(1993).
- Murtaugh, MP et al., "Comparison of the structural protein coding sequences of the VR-2332 and Lelystad vims strains of the PRRS virus," *Arch Virol* 40:1 pp. 1451-1460 (1995).
- Meng, X.J. et al., "Molecular cloning and nucleotide sequencing of the 3'-terminal genomic RNA of the porcine reproductive and respiratory syndrome virus," *J. Gen. Virol.*, 1795-1801(1994).
- Meulenber, J.J.M. et al., *Journal of Virology* 72, 380-387 (1988).
- Murtaugh, EMBL online database, U87392. PRRS virus strain VR-2332 complete genome, Jan. 8, 1998.
- Nelson, CJ, et al, GenBank, AF066183, Porcine reproductive and respiratory syndrome vims Resp PRRS MLV, complete genome, May 15, 1998.
- Remington, "Pharmaceutical Science," 18th ed. Mack Publishing (1990).
- Sambrook, et al., "Molecular Cloning: A Laboratory manual" 2^{ed}., Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY (1989).
- Snijder, E.J. et al., "The molecular biology of arteriviruses," *Journal of General Virology*, 79:961-979 (1998).
- Suarez. P. et al., "Phylogenetic relationships of European strains of porcine reproductive and respiratory syndrome virus (PRRSV) inferred from DNA sequences of putative ORF-5 and ORF-7 genes," *Virus Research*, 42:159-165(1996).
- Terpstra, C. et al., Experimental reproduction of porcine epidemic abortion and respiratory syndrome (mystery swine disease) by infection with Lelystad virus: Koch's postulates fulfilled. *Vet. Quart.*, 13:131-136 (1991).
- Van Dinten LC., et al., An infectious arterivirus cDNA clone: identification of a replicase point mutation, which abolishes discontinuous mRNA transcription. *Proceedings of the National Academy of Science USA* 94: 991-996, (1997).
- Wensvoort, G et al., Mystery swine disease in the Netherlands: the isolation of Lelystad virus, *Vet Quart.*, 13:121-130(1991).
- Yang SX et al., Comparative sequence analysis of open reading frames 2 to 7 of the modified live vaccine virus and other North American isolates of the porcine reproductive and respiratory syndrome virus, *Archives of Virology*, New York, NY, US vol. 143, No. 3, 1998, pp. 601-612.
- Murtaugh, et al., EMBL Online Database, U87392, PRRS virus strain VR-2332 complete genome, Nov. 19, 1998 last update Version 4.
- Choi et al., *Journal of Virology*, 80: 723-736, 2006.
- Nelsen et al., *Journal of Virology*, 73: 270-280, 1999.
- Nielsen et al., *Journal of Virology*, 77: 3702-3711, 2003.

(56)

References Cited

OTHER PUBLICATIONS

- Tan et al., *Virus Genes*, 22: 209-217, 2001.
- Abstract 176 from the CRWAD 82nd Annual Meeting, Nov. 2001.
- Abstract p. 6.6 from the IXth International Symposium on Nidoviruses, May 2003.
- Han et al., *Journal of Virology*, 83: 9449-9463, 2009.
- Posthuma et al., *Journal of Virology*, 82: 4480-4491, 2008.
- Pei et al., *Virology*, 389: 91-99, 2009.
- Kapur et al., *Journal of General Virology*, 77: 1271-1276, 1996.
- Chen, Z., X. Zhou, et al. (2010). "Immunodominant epitopes in nsp2 of porcine reproductive and respiratory syndrome virus are dispensable for replication, but play an important role in modulation of the host immune response." *Journal of General Virology*, 91: 1047-1057.
- Faaberg, K. S., M. E. Kehrli, et al. (2010). "In vivo growth of porcine reproductive and respiratory syndrome virus engineered nsp2 deletion mutants." *Virus Research*, 154: 77-85.
- Guo, B., A. C. Vorwald, et al. (2011). "Large scale parallel pyrosequencing technology: PRRSV strain VR-2332 nsp2 deletion mutant stability in swine." *Virus Research*, 161: 162-169.
- Vandeputte et al., *American Journal of Veterinary Research*, 2001; 62: 1805-1811.
- Murtaugh et al., *Vaccine*, 2011; 29: 8192-8204.
- Issued Patents_NA database sequence alignment of U.S. Pat. No. 6,500,662 SEQ ID No. Dec. 1, 2002 with SEQ ID No. 6.
- Issued Patents_NA database sequence alignment of U.S. Pat. No. 7,132,106 SEQ ID No. Nov. 1, 2006 with SEQ ID No. 6.
- Issued Patents_NA database sequence alignment of U.S. Pat. No. 7,232,680 SEQ ID No. Jun. 1, 2007 with SEQ ID No. 6.
- Issued Patents_NA database sequence alignment of U.S. Pat. No. 7,618,797 Nov. 2009 SEQ ID No. 1 with SEQ ID No. 6.
- Issued Patents_NA database sequence alignment of U.S. Pat. No. 7,691,389 Apr. 2010 SEQ ID No. 1 with SEQ ID No. 6.
- Issued Patents_NA database sequence alignment of U.S. Pat. No. 8,481,705 Jul. 2013 SEQ ID No. 1 with SEQ ID No. 6.
- Issued Patents_NA database sequence alignment of U.S. Pat. No. 8,492,132 Jul. 2013 SEQ ID No. 1 with SEQ ID No. 6.
- Published_Applications_NA_Main database sequence alignment of U.S. Appl. No. 12/615,504, now U.S. Pat. No. 8,609,827 Dec. 2013 SEQ ID No. 1 with SEQ ID No. 6.
- Published_Applications_NA_Main database sequence alignment of U.S. Appl. No. 12/608,305 SEQ ID No. 1 with SEQ ID No. 6.
- SEQ ID No. 6 sequence alignment with Genseq AFR07575, Jun. 2007.
- GenEmbl ID GP721394 sequence alignment with SEQ ID No. 6, Dec. 2009.
- Colson et al., *Applied Animal Behavior Science*, 2006, vol. 98, pp. 70-88.
- O'Neill et al., *Clinical and Vaccine Immunology*, Nov. 2011, vol. 18, pp. 1865-1871.
- Reynolds et al., *The Veterinary Journal*, 2009, vol. 181, pp. 312-320.
- Murtaugh et al., *Virus Research*, 2010, vol. 154, pp. 18-30.
- Savard et al., *Canadian Journal of Veterinary Research*, 2016, vol. 80, pp. 1-11.
- Han et al., *Veterinary Microbiology*, 2017, vol. 209, pp. 30-47.
- Jeong et al., *Veterinary Microbiology*, 2018, vol. 214, pp. 113-124.
- O'Brien et al., "Fostera PRRS Vaccine for One-Day-Old Piglets: Respiratory Efficacy, Duration of Immunity, and Safety Research", Dec. 2014, vol. 1-8, retrieved on internet at: <https://www.Zoetisus.com/products/pork/fosteraprrs/documents/tech-update-fostera-prrs-1-day-of-age.pdf>.
- Zhou et al., *Veterinary Microbiology*, 2017, vol. 207, pp. 108-116.
- Balasch, M. et al., 2018, "Vaccination of 1-day-old pigs with a porcine reproductive and respiratory syndrome virus (PRRSV) modified live attenuated virus vaccine is able to overcome maternal immunity", *Porcine Health Management*, vol. 4, pp. 1-11.
- Pearce, D. S. et al., 2014, "Live virus determination of PRRSV vaccines on primary porcine alveolar macrophages", *Proceedings 23rd International Pig Veterinary Society*, p. 162, Jun. 8-11, 2014. Cancun, Mexico.
- Xiangju Wu et al., "Establishment and Characterization of a High and Stable Porcine CD163-Expressing MARC-145 Cell Line", *BioMed Research International*, Article ID 4315861, 9 pages, Hindawi Publishers, 2018, <https://doi.org/10.1155/2018/4315861>.
- Sornsen, S. A. et al., "Effect of various stocking methods and extra-label PRRS vaccination on average daily gain", *Swine Health and Production*, 1998, vol. 6, pp. 7-11.

* cited by examiner

Figure 1

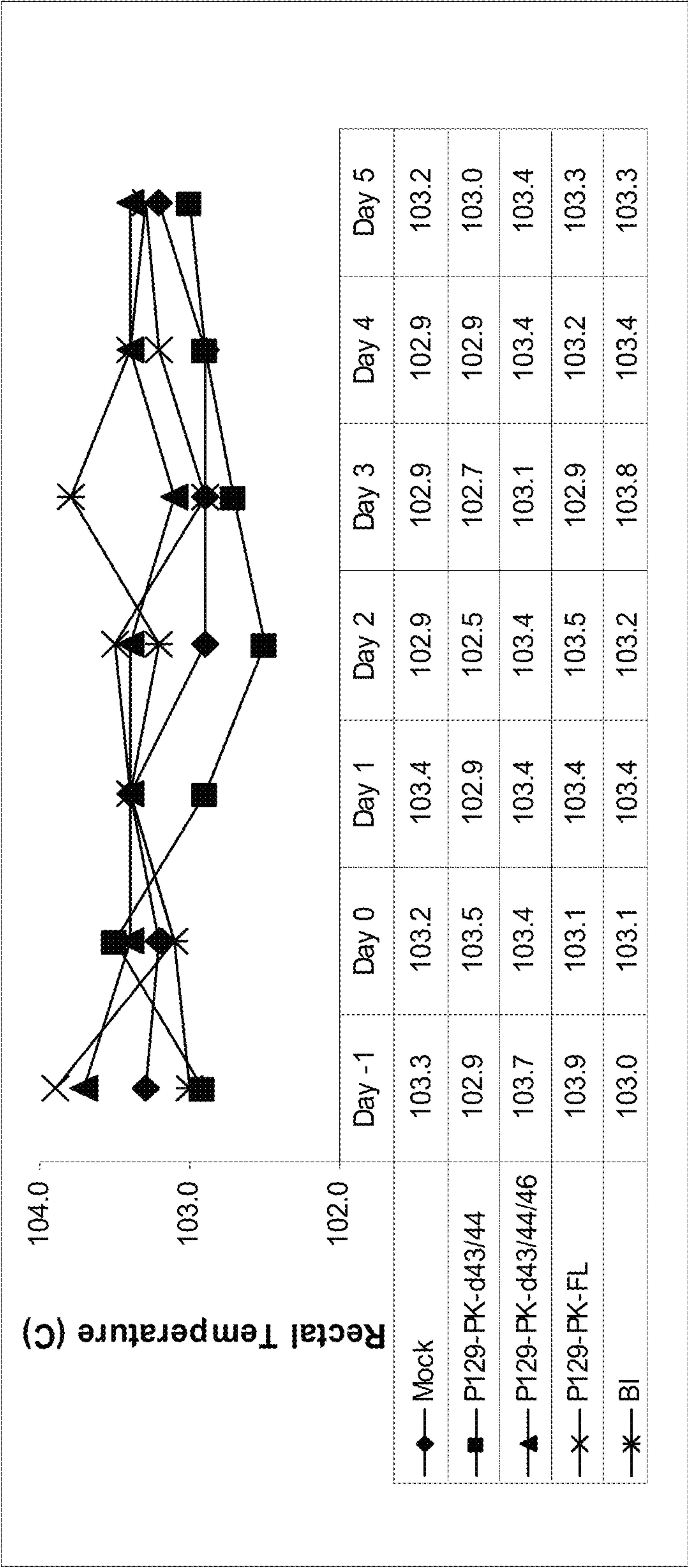


Figure 2

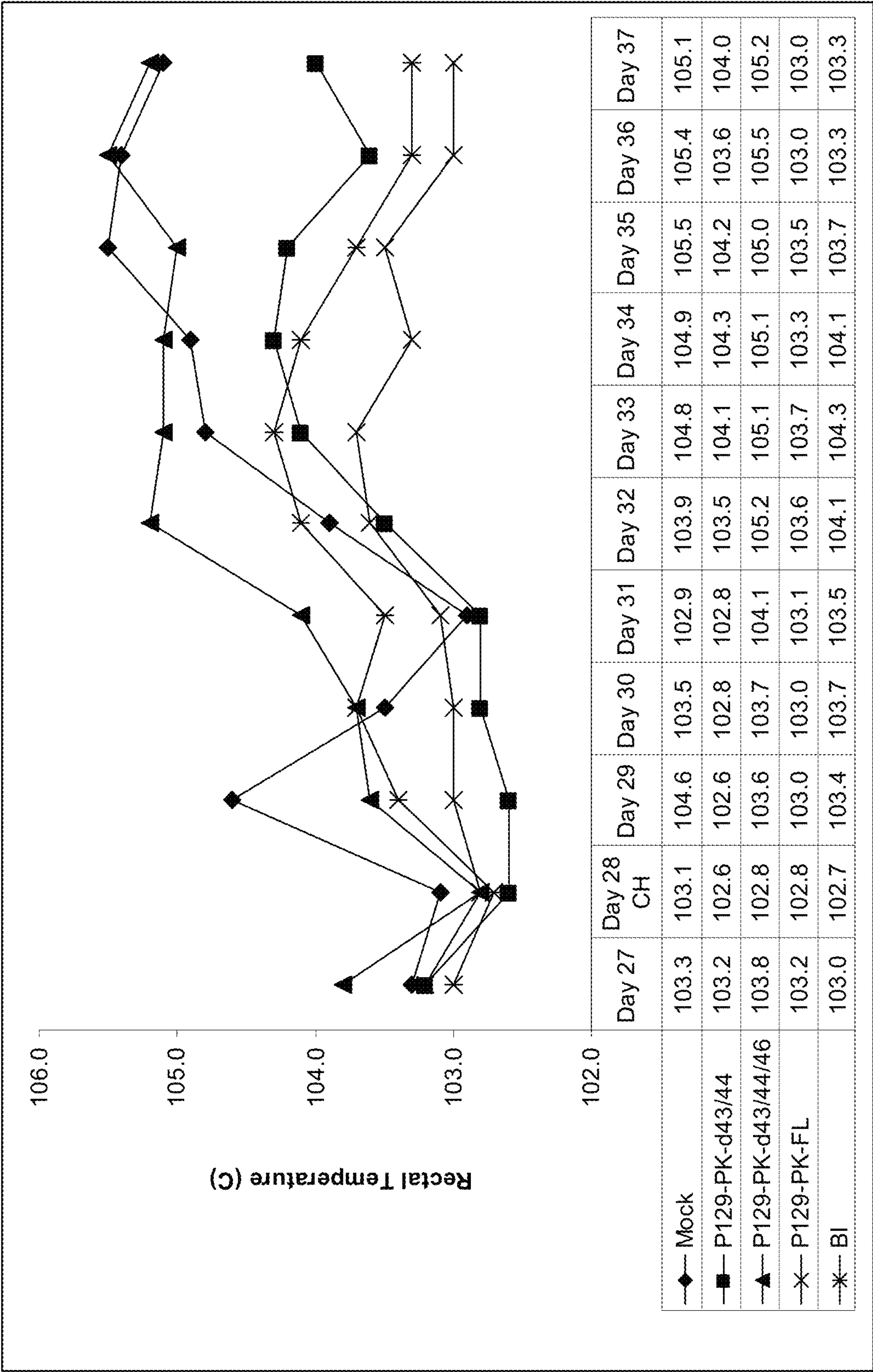


Figure 3

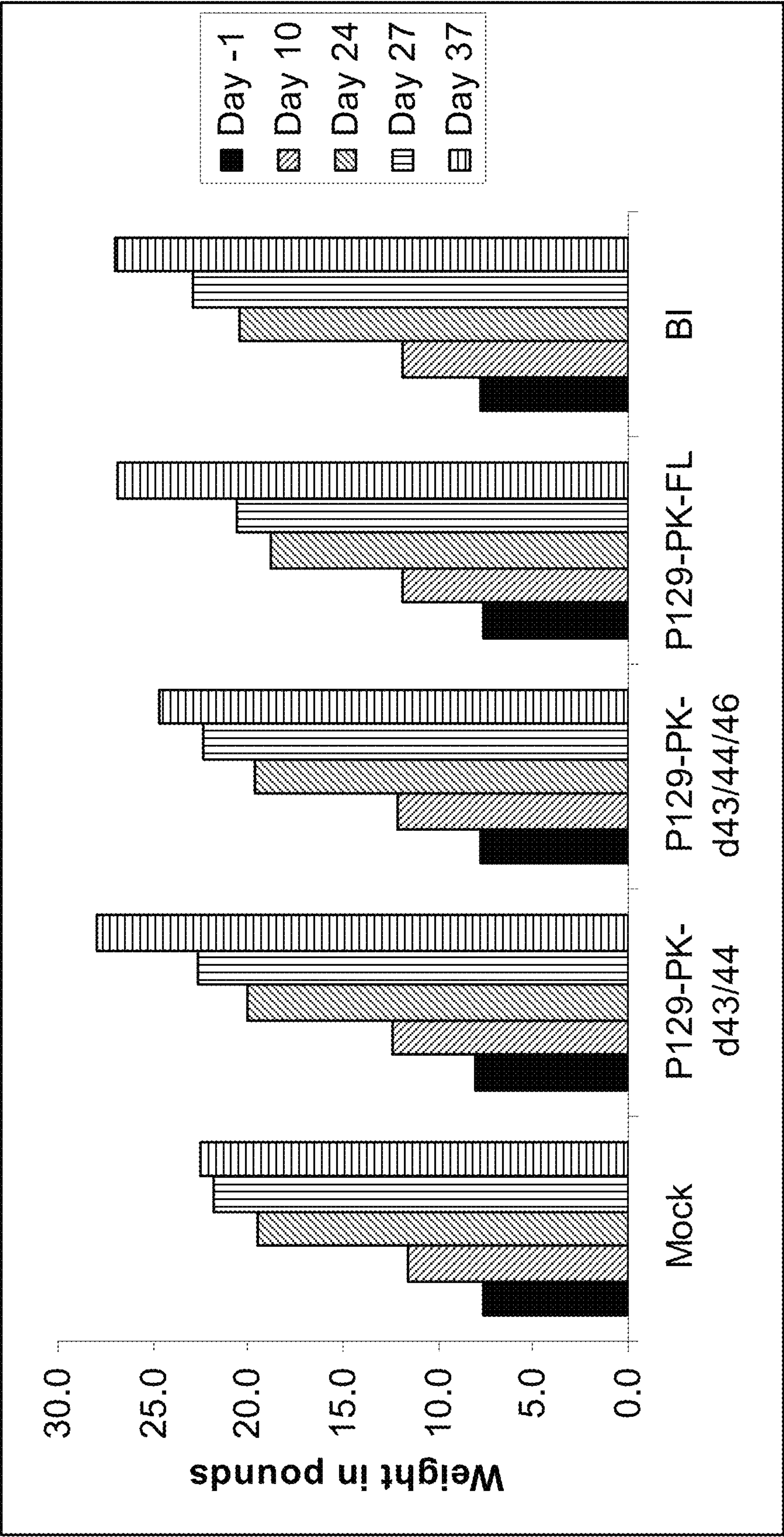


Figure 4

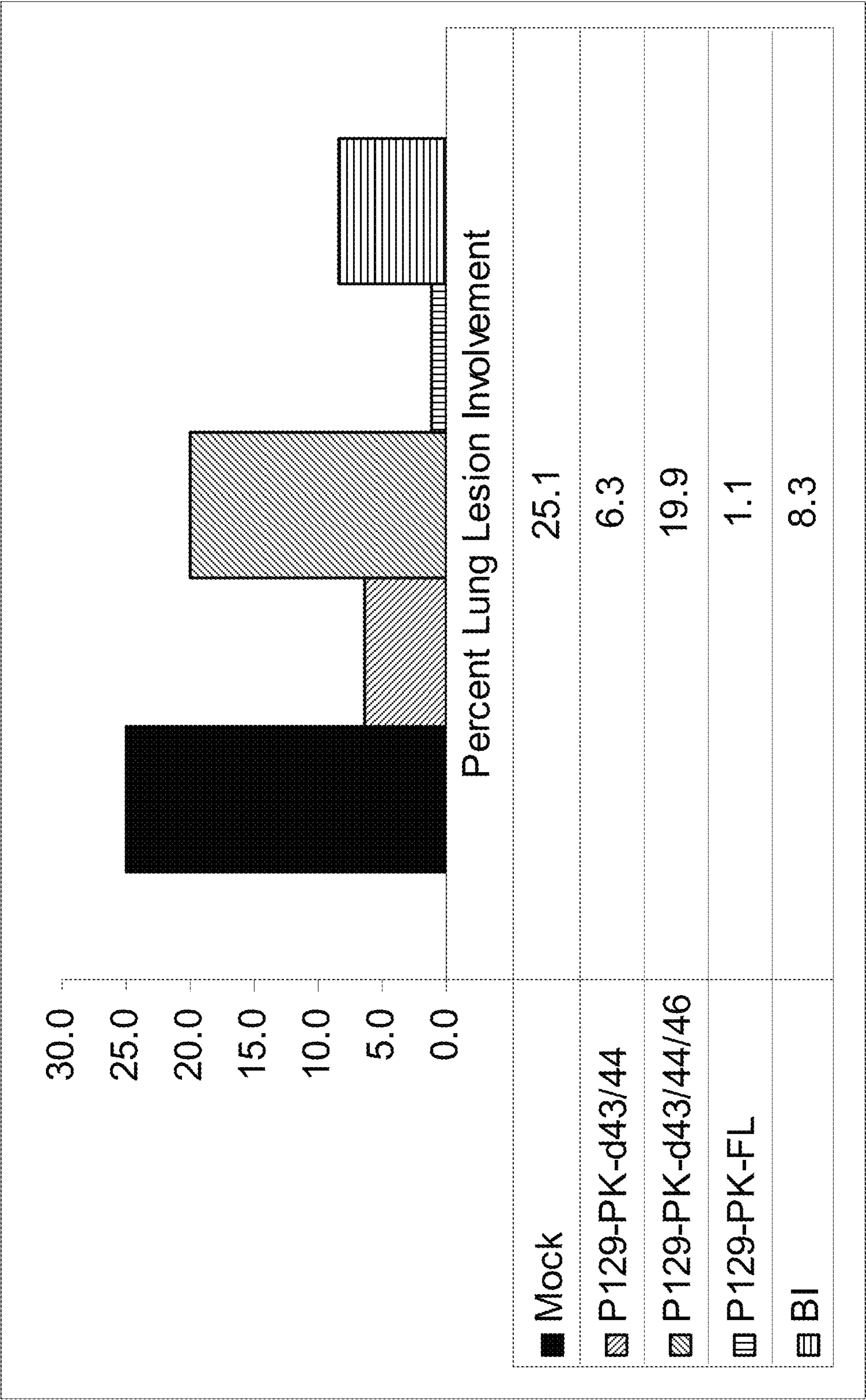


Figure 5

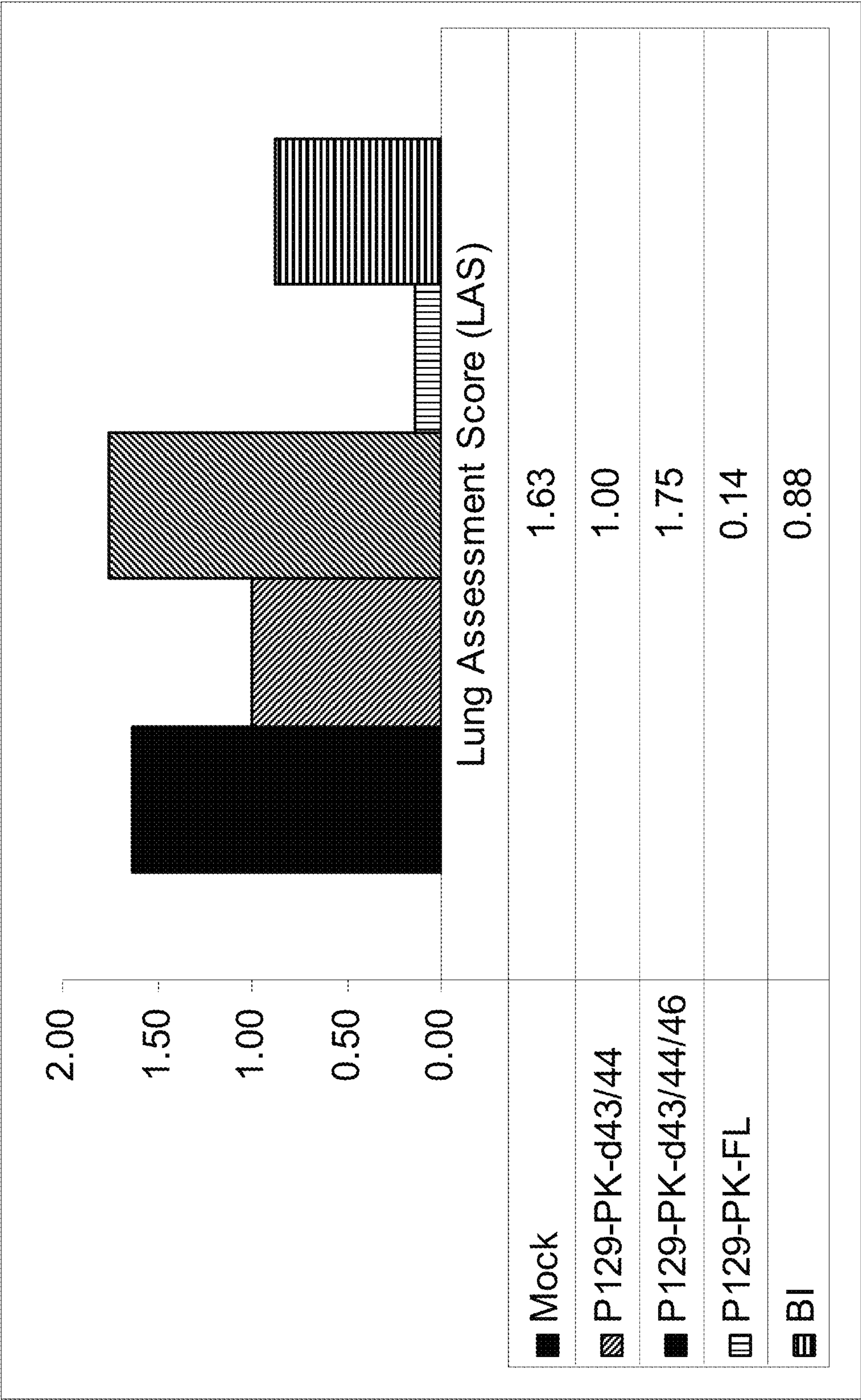


Figure 6

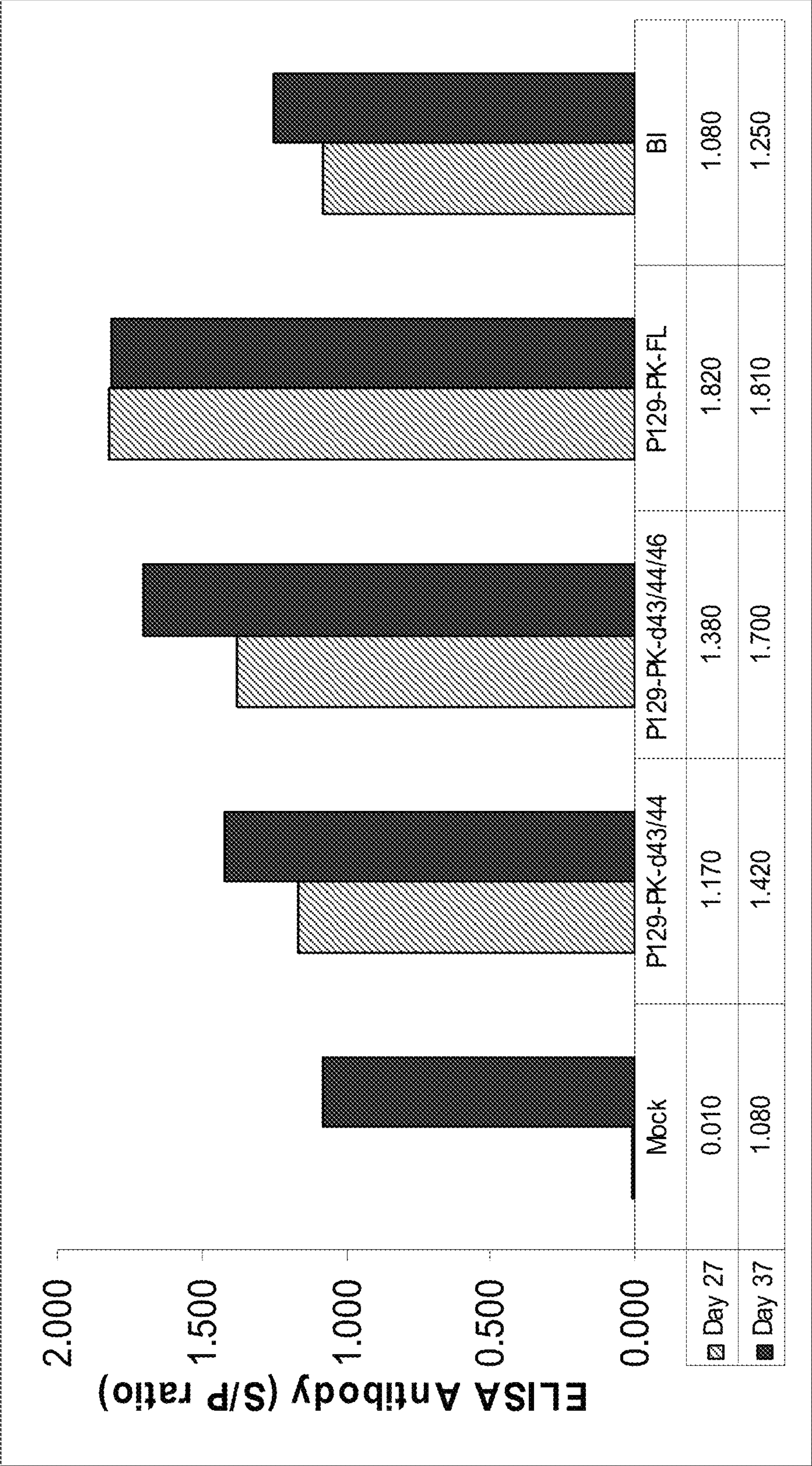


Figure 7

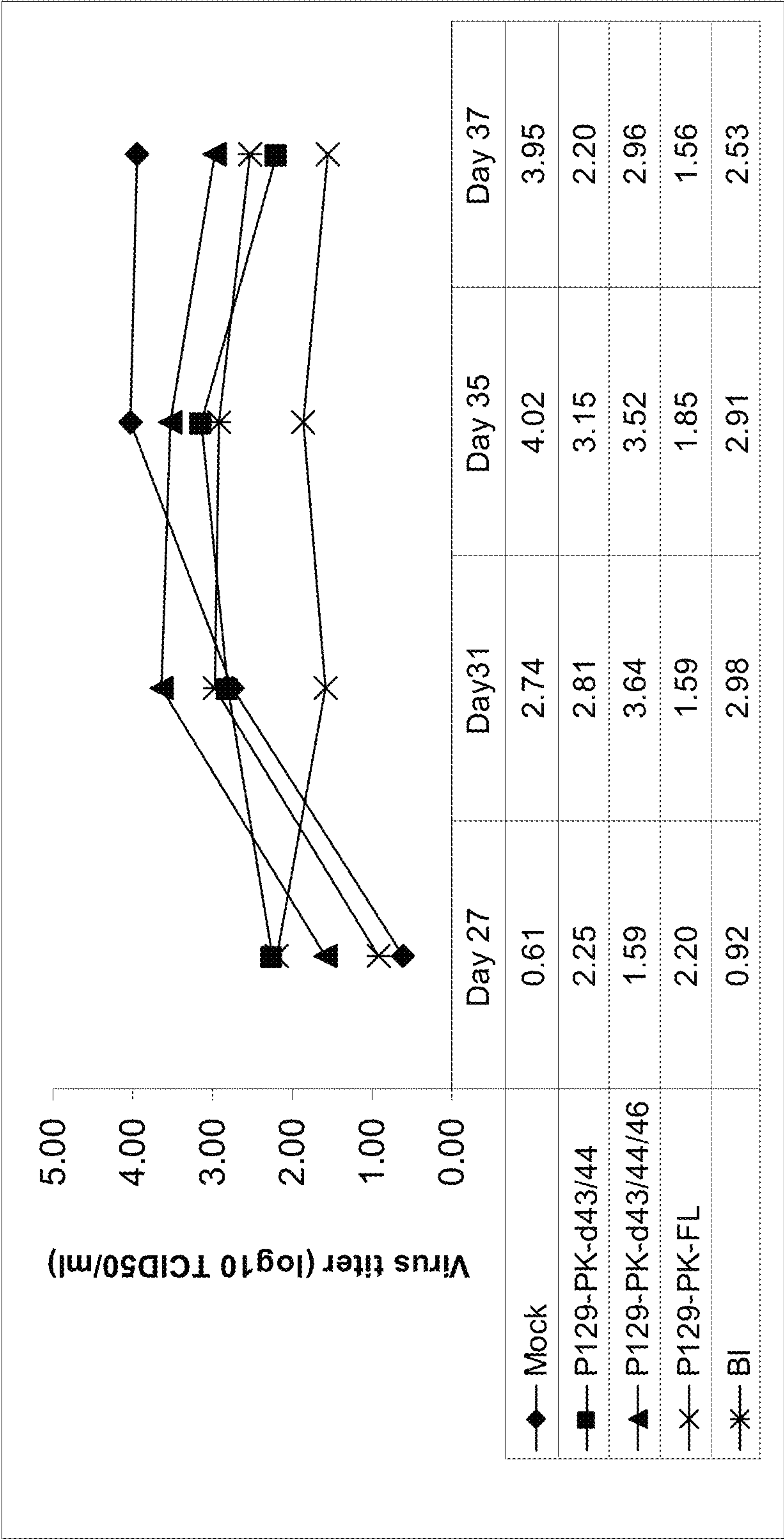
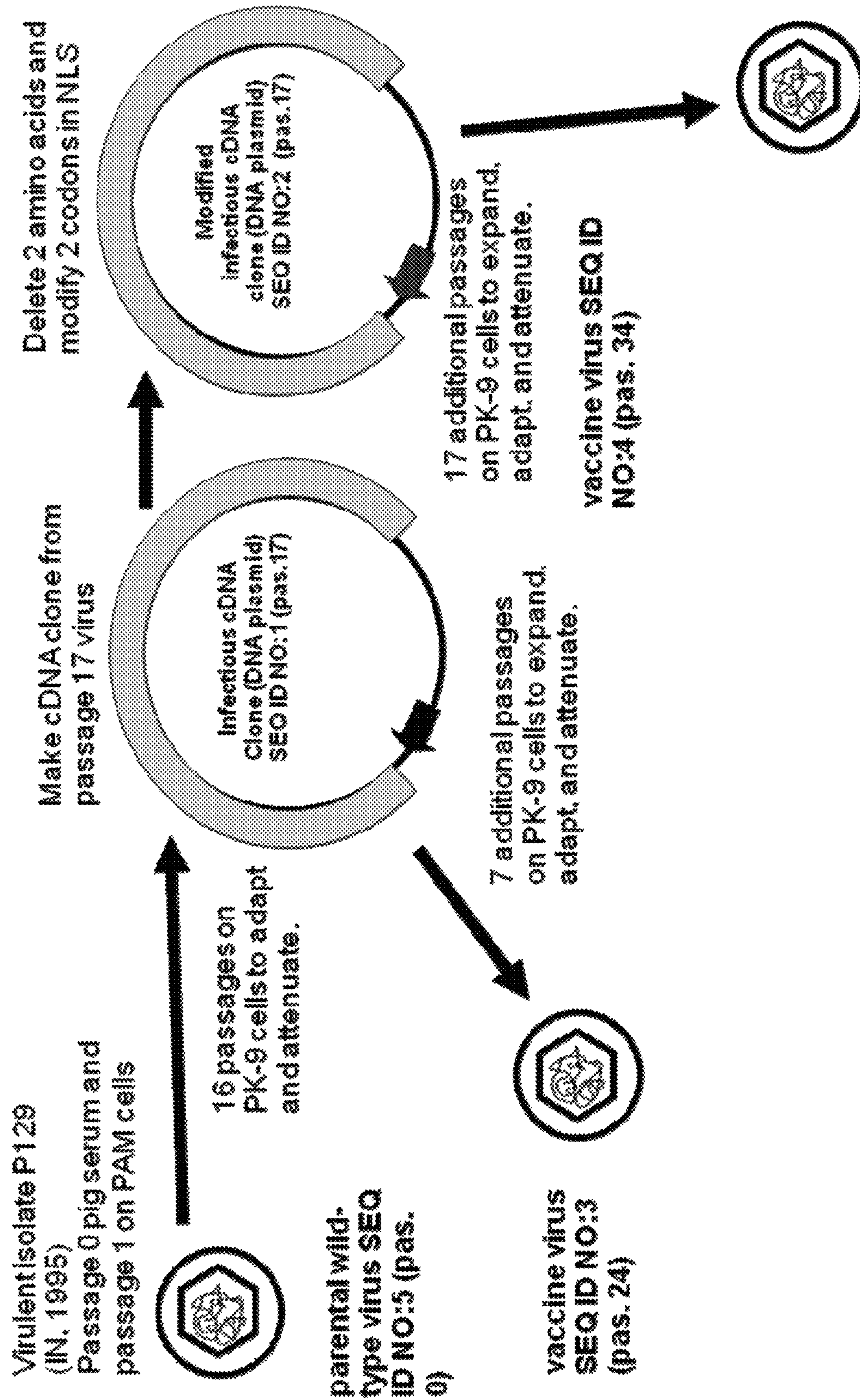


Figure 8



1

EFFECTIVE VACCINATION AGAINST PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) VIRUS PRIOR TO WEANING

The present application is a continuation of U.S. application Ser. No. 13/895,564, filed May 16, 2013, and claims the benefit under 35 USC 119(e) of U.S. provisional application 61/648,461, filed May 17, 2012.

FIELD OF THE INVENTION

The present invention is in the field of animal health and is directed to infectious cDNA clones of positive polarity RNA viruses, novel RNA viruses and modified live forms thereof, and the construction of vaccines, in particular, swine vaccines, using such cDNA clones. More particularly, the present invention also provides for the safe and early vaccination of piglets prior to weaning, including from immediately after birth (i.e. only 1 day of age or less) to two weeks of age, at all times optionally in combination with multivalent combination swine vaccines, such as bivalent PRRSV/*Mycoplasma hyopneumoniae* (*M. hyo*) vaccines, bivalent PRRSV/Porcine Circovirus type 2 (PCV2) vaccines, and trivalent PRRSV/*M. hyo*/PCV2 vaccines, or simply as a monovalent PRRSV vaccine. Early vaccination against PRRS under such conditions provides an early onset of protective immunity, that arises no later than about 14 days after vaccination, i.e. at Day 15 following vaccination on Day 1 of life, Day 21 following vaccination on Day 7 of life, and no later than about Day 28 following vaccination on Day 14 of life. Although the present specification provides for numerous constructs of the "P129 strain" of North American PRRS virus (see PCT/1B2011/055003 and U.S. Pat. No. 6,500,662), which are highly effective as vaccines, including for such early and safe use, it has been determined that such early onset of protective immunity (i.e. about 2 weeks following immunizing vaccination given as early as Day 1 after birth), is also applicable to use of other North American and European PRRS strains, such as those described in U.S. Pat. Nos. 5,476,778, 5,846,805, 6,380,376, 6,982,160 and 6,197,310.

BACKGROUND OF THE INVENTION

Porcine reproductive and respiratory syndrome (PRRS) is characterized by abortions, stillbirths, and other reproductive problems in sows and gilts, as well as respiratory disease in young pigs. The causative agent is the PRRS virus (PRRSV), a member of the family Arteriviridae and the order Nidovirales. The nidoviruses are enveloped viruses having genomes consisting of a single strand of positive polarity RNA. The genomic RNA of a positive-stranded RNA virus fulfills the dual role in both storage and expression of genetic information. No DNA is involved in replication or transcription in Nidoviruses. The non-structural proteins are translated directly from the genomic RNA of nidoviruses as large polyproteins and subsequently cleaved by viral proteases into discreet functional proteins. A 3'-co-terminal nested set of subgenomic RNAs (sgRNAs) is synthesized from the genome and are used as messenger RNAs for translation of the structural proteins. The reproduction of nidoviral genomic RNA is thus a combined process of genome replication and sgRNA synthesis.

In the late 1980's, two distinct genotypes of the virus emerged nearly simultaneously, one in North America and another in Europe. PRRS virus is now endemic in nearly all

2

swine producing countries, and is considered one of the most economically important diseases affecting the global pork industry. Additionally, highly virulent genotypes have been isolated in China and surrounding countries, and such genotypes are generally related to North American genotypes.

Despite significant advances in understanding the biology of PRRSV, control of the virus remains difficult. Vaccination of animals in the field has proven to be largely ineffective. PRRS commonly re-emerges in immunized herds, and most on-farm PRRSV vaccination campaigns ultimately fail to control the disease.

Without being limited as to theory, infection of pigs with wild type PRRSV or their vaccination with a live attenuated form of this pathogen unfortunately only elicits an exuberant production of non-neutralizing antibodies. During this time interval, for example, only limited quantities of interferon (IFN)- γ (secreting cells are generated. Thus, PRRSV seems to inherently stimulate an imbalanced immune response distinguished by consistently abundant humoral (antibody-based) immunity, and a variable and limited but potentially protective T helper (Th) 1-like IFN- γ response. One characteristic of PRRSV infection that most likely contributes to the imbalanced development of adaptive immunity is the lack of an adequate innate immune response. Usually, virus-infected cells secrete type I interferon "IFN" (including IFN- α and IFN- β), which protects neighboring cells from infection. In addition, the released type I IFN interacts with a subset of naïve T cells to promote their conversion into virus-specific type II IFN (IFN- γ) secreting cells. In contrast, the IFN- α response of pigs to PRRSV exposure is nearly non-existent. Such inefficient stimulation of IFN- α production by a pathogen would be expected to have a significant impact on the nature of the host's adaptive immune response, since IFN- α up-regulates IFN- γ gene expression. Accordingly, the former cytokine controls the dominant pathway that promotes the development of adaptive immunity, namely, T cell-mediated IFN- γ responses and peak antiviral immune defenses.

In this regard, it has become evident that a probable link between innate and adaptive immunity in viral infections occurs through a special type of dendritic cell which has the ability to produce large amounts of type I interferon, and which plays a critical role in the polarization of T-cell function. Specifically, an infrequent but remarkable type of dendritic cell, the plasmacytoid dendritic cell (PDC), also known as a natural IFN- α/β -producing cell, plays a critical role in anti-viral immunity by means of their ability to cause naïve T cells to differentiate into IFN- γ secreting cells. Although rare, the PDC are enormously potent producers of IFN- α , with each cell being capable of producing 3-10 pg of IFN- α in response to virus. In contrast, monocytes produce 5- to 10-fold less IFN- α on a per cell basis. The phenotype and some biological properties of porcine PDC have been described (Summerfield et al., 2003, Immunology 110:440). Recent studies have determined that PRRSV does not stimulate porcine PDCs to secrete IFN- α (Calzada et al., 2010, Veterinary Immunology and Immunopathology 135:20).

This fact, in combination with the observation that exogenously added IFN- α at the time of vaccination has been found to improve the intensity of the PRRSV-specific IFN- γ response (W. A. Meier et al., Vet. Immunol. Immunopath. 102, pp 299-314, 2004), highlights the critical role that IFN- α plays during the infection of pigs with this virus. Given the apparent critical role of IFN- α on the development of protective immunity, it is important to determine the ability of different PRRS virus stocks to stimulate and/or inhibit the production of IFN- α . Accordingly, there is a

pressing need for new and improved modified live vaccines to protect against PRRS. As described below, it is clear that viruses derived from the novel infectious cDNA clone, pCMV-S-P129-PK, and others, have a different phenotype than either the wild-type P129 virus or two commercially available modified live PRRS vaccines. Without being limited as to theory, the present invention provides for vaccines that facilitate cell-based immune response against the virus, and define a new and effective generation of PRRS vaccines.

SUMMARY OF THE INVENTION

In a first embodiment, the present invention provides an isolated polynucleotide molecule including a DNA sequence encoding an infectious RNA molecule encoding a PRRS virus that is genetically modified such that, as a vaccine, it elicits an effective immunoprotective response against the PRRS virus in porcine animals. In certain aspects, the invention provides for a DNA sequence as set forth herein including SEQ ID NO.:1, SEQ ID NO.:2, SEQ ID NO.:3, SEQ ID NO.:4, or SEQ ID NO.:6, or a sequence having at least 70% identity thereto, preferably 80% identity thereto, and more preferably 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereto.

In certain embodiments, the invention provides for a plasmid that includes an isolated polynucleotide molecule as set forth herein and a promoter capable of transcribing the polynucleotide molecule in a suitable host cell. In another embodiment, the North American or Chinese PRRS encoding sequence of the plasmid herein further encodes one or more detectable heterologous antigenic epitopes. The present invention provides for a transfected host cell that includes the plasmid set forth herein.

In another aspect, the present invention provides for a vaccine for protecting a porcine animal from infection by a PRRS virus. The vaccine may include a North American or Chinese PRRS virus encoded by an infectious RNA molecule, the infectious RNA molecule, or a plasmid, each of which are encoded by the isolated polynucleotide molecule as set forth herein. In yet another aspect, the vaccine includes a viral vector including the polynucleotide herein. The vaccine set forth herein may optionally include a vaccine carrier acceptable for veterinary use. In one important aspect, the vaccine has a decreased interferon- α inhibitory effect as compared to wild-type P129 PRRS virus (see ATCC 203488, 203489, U.S. Pat. No. 6,500,662).

In one embodiment, the present invention provides for diagnostic kit including polynucleotide molecules which distinguish (a so-called DIVA test) between porcine animals naturally infected with a field strain of a PRRS virus and porcine animals vaccinated with the modified live vaccine set forth herein.

In other embodiments, the invention provides for a method of protecting a porcine animal from infection with a strain of PRRS virus including administering to the animal an immunogenically protective amount of the vaccine of the claims set forth herein.

Further and preferred embodiments of the invention include an isolated Porcine Reproductive and Respiratory Syndrome Virus (PRRS), or a polynucleotide sequence encoding therefor, wherein the protein encoded by ORF1a is selected from a group consisting of those that contain any of the following amino acid sequences, wherein the underlined residues are believed to be novel: AMANVYD (SEQ ID NO: 9); IGHNAVM (SEQ ID NO: 12); TVPDGNC (SEQ ID NO: 15); CWWYLFD (SEQ ID NO: 18); HGVHGKY (SEQ ID NO: 21); AAKVDQY (SEQ ID NO: 24); PSATDTS

(SEQ ID NO: 27); LNSLLSK (SEQ ID NO: 30); APM CQDE (SEQ ID NO: 33); CAPTGMD (SEQ ID NO: 36); PKVAKVS (SEQ ID NO: 39); AGEIVGV (SEQ ID NO: 42); ADFNPEK (SEQ ID NO: 45); and QTPILGR (SEQ ID NO: 48). In a further preferred embodiment of the invention, the invention provides an isolated North American or Chinese PRRS that contain any of the above-identified sequences within the protein encoded from ORF1a, including any combinations (2, 3, 4 . . . up to 17) of these identified sequences.

The invention further provides for an isolated Porcine Reproductive and Respiratory Syndrome Virus (PRRS) wherein the protein thereof encoded by ORF1a is selected from a group consisting of those amino acid sequences that contain any of: ANV (see SEQ ID NO: 9); HNA (see SEQ ID NO: 12); PDG (see SEQ ID NO: 15); WYL (see SEQ ID NO: 18); VHG (see SEQ ID NO: 21); KVD (see SEQ ID NO: 24); ATD (see SEQ ID NO: 27); SLL (see SEQ ID NO: 30); MCQ (see SEQ ID NO: 33); PTG (see SEQ ID NO: 36); VAK (see SEQ ID NO: 39); EIV (see SEQ ID NO: 42); FNP (see SEQ ID NO: 45); and PIL (see SEQ ID NO: 48), including any combinations (2, 3, 4 . . . up to 17) of these identified sequences.

In a further preferred embodiment, the invention provides an isolated North American or Chinese PRRS wherein, irrespective of the identity of any other specific nucleotide or amino acid sequence positions at any point in a polynucleotide encoding the virus or the proteins encoded therefrom, the ORF1a virus protein contains:

- (a) any of the following specific amino acids in the specified sequences,
 - an amino acid N within the amino acid sequence ANV (see SEQ ID NO: 9);
 - an amino acid N within the amino acid sequence HNA (see SEQ ID NO: 12);
 - an amino acid D within the amino acid sequence PDG (see SEQ ID NO: 15);
 - an amino acid Y within the amino acid sequence WYL (see SEQ ID NO: 18);
 - an amino acid H within the amino acid sequence VHG (see SEQ ID NO: 21);
 - an amino acid V within the amino acid sequence KVD (see SEQ ID NO: 24);
 - an amino acid T within the amino acid sequence ATD (see SEQ ID NO: 27);
 - an amino acid L within the amino acid sequence SLL (see SEQ ID NO: 30);
 - an amino acid C within the amino acid sequence MCQ (see SEQ ID NO: 33);
 - an amino acid T within the amino acid sequence PTG (see SEQ ID NO: 36);
 - an amino acid A within the amino acid sequence VAK (see SEQ ID NO: 39);
 - an amino acid I within the amino acid sequence EIV (see SEQ ID NO: 42);
 - an amino acid N within the amino acid sequence FNP (see SEQ ID NO: 45); and
 - amino acid I within the amino acid sequence PIL (see SEQ ID NO: 48), to include any combinations (2, 3, 4 . . . up to 17) of these identified sequences, or
- (b) contains said specific underlined single amino acids in the specified 3-residue ORF1a peptide sequences of any other North American or Chinese PRRS viruses that correspond to the 3-residue sequences as specified above, taking into account that said other specific 3-residue amino acid sequences may show one or two additional amino sequence changes, but still be recognized as corresponding to the

5

sequences specified above. For the purposes of this embodiment of the invention, "corresponding" means that the relative sequences can be optimally aligned using a BLOSUM algorithm as described in Henikoff et al. Proc Natl. Acad. Sci., USA, 89, pp. 10915-10919, 1992.

In a further preferred embodiment of the invention, an isolated Porcine Reproductive and Respiratory Syndrome Virus (PRRS) is provided wherein the protein thereof encoded by ORF1a has an amino acid sequence that contains one or more of variations (a), (b), (c) and (d), wherein each said variation is defined as follows:

variation (a),

an amino acid N within the amino acid sequence ANV (see SEQ ID NO: 9);

an amino acid N within the amino acid sequence HNA (see SEQ ID NO: 12);

an amino acid D within the amino acid sequence PDG (see SEQ ID NO: 15),

an amino acid Y within the amino acid sequence WYL (see SEQ ID NO: 18);

an amino acid H within the amino acid sequence VHG (see SEQ ID NO: 21), or any subset of variation (a);

variation (b),

an amino acid V within the amino acid sequence KVD (see SEQ ID NO: 24);

an amino acid T within the amino acid sequence ATD (see SEQ ID NO: 27);

an amino acid L within the amino acid sequence SLL (see SEQ ID NO: 30).

an amino acid C within the amino acid sequence MCQ (see SEQ ID NO: 33), or any subset of variation (b);

variation (c),

an amino acid T within the amino acid sequence PTG (see SEQ ID NO: 36);

an amino acid A within the amino acid sequence VAK (see SEQ ID NO: 39), or any subset of variation (c); and variation (d),

an amino acid I within the amino acid sequence EIV (see SEQ ID NO: 42);

an amino acid N within the amino acid sequence FNP (see SEQ ID NO: 45);

and amino acid I within the amino acid sequence PIL (see SEQ ID NO: 20), or any subset of variation (d) thereof.

Such PRRS viruses may further contain two or more of the five amino acid sequences identified in variation (a), and/or two or more of the four amino acid sequences identified in variation (b), and/or the two amino acid sequences identified in variation (c), and/or two or more of the three amino acid sequences identified in variation (d).

The present invention also provides a plasmid capable of directly transfecting a suitable host cell and expressing a Porcine Reproductive and Respiratory Syndrome Virus (PRRS) from the suitable host cell so transfected, which plasmid comprises: (a) a DNA sequence encoding an infectious RNA molecule encoding the PRRS virus, and (b) a promoter capable of transcribing said infectious RNA molecule, wherein the protein encoded by ORF1a of said virus has an amino acid sequence that contains:

(1) an amino acid N within the amino acid sequence ANV (see SEQ ID NO: 9);

an amino acid N within the amino acid sequence HNA (see SEQ ID NO: 12);

an amino acid D within the amino acid sequence PDG (see SEQ ID NO: 15),

an amino acid Y within the amino acid sequence WYL (see SEQ ID NO: 18);

6

an amino acid H within the amino acid sequence VHG (see SEQ ID NO: 21), or any subset thereof; and/or

(2) an amino acid V within the amino acid sequence KVD (see SEQ ID NO: 24);

5 an amino acid T within the amino acid sequence ATD (see SEQ ID NO: 27);

an amino acid L within the amino acid sequence SLL (see SEQ ID NO: 30).

10 an amino acid C within the amino acid sequence MCQ (see SEQ ID NO: 33), or any subset thereof; and/or

(3) an amino acid T within the amino acid sequence PTG (see SEQ ID NO: 36);

15 an amino acid A within the amino acid sequence VAK (see SEQ ID NO: 39), or any subset thereof; and/or

(4) an amino acid I within the amino acid sequence EIV (see SEQ ID NO: 42);

an amino acid N within the amino acid sequence FNP (see SEQ ID NO: 45);

20 and amino acid I within the amino acid sequence PIL (see SEQ ID NO: 48), or any subset thereof.

It will be appreciated that ORF1a encodes a polyprotein comprising protease function, and ORF1b encodes a polyprotein comprising replicase (RNA polymerase) and helicase functions. Additional information concerning the functions for proteins encoded from various ORFs (open reading frames) of PRRS may be found, for example, in U.S. Pat. No. 7,132,106. See also U.S. Pat. No. 7,544,362 in regard of function of ORF7, and other open reading frames. As would be appreciated in the art, the ORF1-encoded proteins are expected to have additional functions, known and unknown, and the novel amino acid changes useful in the practice of the present invention are not limited via their effects on any one specific function of the ORF1-encoded proteins.

35 In further preferred embodiments, said plasmid contains a promoter that is a eukaryotic promoter capable of permitting a DNA launch in targeted eukaryotic cells, or a prokaryotic or phage promoter capable of directing in vitro transcription of the plasmid. The invention similarly provides a method of generating a PRRS virus, which method comprises transfecting a suitable host cell with an appropriate plasmid and obtaining PRRS virus generated by the transfected cell.

Accordingly, in a specific and preferred embodiment, the invention provides an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus, wherein said DNA sequence is selected from the group consisting of:

(a) SEQ ID NO:6;

45 (b) a sequence that has at least 85% identity to the DNA sequence of (a) wherein the protein encoded by ORF1a thereof has an amino acid sequence that contains:

from group (b) (1)

an amino acid N within the amino acid sequence ANV (see SEQ ID NO: 9);

55 an amino acid N within the amino acid sequence HNA (see SEQ ID NO: 12);

an amino acid D within the amino acid sequence PDG (see SEQ ID NO: 15),

an amino acid Y within the amino acid sequence WYL (see SEQ ID NO: 18);

an amino acid H within the amino acid sequence VHG (see SEQ ID NO: 21), or any subset thereof; and/or

from group (b) (2)

65 an amino acid V within the amino acid sequence KVD (see SEQ ID NO: 24);

an amino acid T within the amino acid sequence ATD (see SEQ ID NO: 27);

an amino acid L within the amino acid sequence SLL (see SEQ ID NO: 30);
 an amino acid C within the amino acid sequence MCQ (see SEQ ID NO: 33), or any subset thereof; and/or
 from group (b)(3)
 an amino acid T within the amino acid sequence PTG (see SEQ ID NO: 36);
 an amino acid A within the amino acid sequence VAK (see SEQ ID NO: 39), or any subset thereof; and/or
 from group (b)(4)
 an amino acid I within the amino acid sequence EIV (see SEQ ID NO: 42);
 an amino acid N within the amino acid sequence FNP (see SEQ ID NO: 45);
 and amino acid I within the amino acid sequence PIL (see SEQ ID NO: 20), or any subset thereof; and
 (c) a DNA sequence that hybridizes to the complement of a DNA sequence of (a) or
 (b) under highly stringent conditions which comprise hybridization to filter bound DNA in 0.5 M NaHPo₄, 7% SDS, 1 mM EDTA at 65 degrees C., and washing in 0.1 SSC/0/1% SDS at 68 degrees C.

The invention also provides for host cells transfected with polynucleotide molecules and provides vaccines for protecting a porcine animal against infection by a PRRS virus, which vaccine comprises: (a) a genetically modified North American PRRS virus encoded by such aforementioned polynucleotide molecules, or (b) said infectious molecule, or (c) said polynucleotide molecule in the form of a plasmid, or (d) a viral vector comprising said polynucleotide molecule, wherein the PRRS virus is able to elicit an effective immunoprotective response against infection by PRRS virus, in an amount effective to produce immunoprotection against infection, and a carrier suitable for veterinary use.

The invention also provides RNA polynucleotide sequences corresponding to (i.e. by having complementary base coding sequences):

(a) the DNA sequence of SEQ ID NO:6;
 (b) a DNA sequence that has at least 85% identity to the DNA sequence of (a) wherein the protein encoded by ORF1a thereof has an amino acid sequence that contains any of the following, and any combination of any of the following:

an amino acid N within the amino acid sequence ANV (see SEQ ID NO: 9);
 an amino acid N within the amino acid sequence HNA (see SEQ ID NO: 12);
 an amino acid D within the amino acid sequence PDG (see SEQ ID NO: 15),
 an amino acid Y within the amino acid sequence WYL (see SEQ ID NO: 18);
 an amino acid H within the amino acid sequence VHG (see SEQ ID NO: 21),
 an amino acid V within the amino acid sequence KVD (see SEQ ID NO: 24);
 an amino acid T within the amino acid sequence ATD (see SEQ ID NO: 27);
 an amino acid L within the amino acid sequence SLL (see SEQ ID NO: 30).
 an amino acid C within the amino acid sequence MCQ (see SEQ ID NO: 33),
 an amino acid T within the amino acid sequence PTG (see SEQ ID NO: 36);
 an amino acid A within the amino acid sequence VAK (see SEQ ID NO: 39),
 (an amino acid I within the amino acid sequence EIV (see SEQ ID NO: 42);

an amino acid N within the amino acid sequence FNP (see SEQ ID NO: 45);
 and amino acid I within the amino acid sequence PIL (see SEQ ID NO: 20), or

(c) a DNA sequence that hybridizes to the complement of a DNA sequence of (a) or (b) under highly stringent conditions which comprise hybridization to filter bound DNA in 0.5 M NaHPo₄, 7% SDS, 1 mM EDTA at 65 degrees C., and washing in 0.1 SSC/0/1% SDS at 68 degrees C.

Accordingly, the invention also provides diagnostic kits comprising polynucleotide molecules which distinguish between porcine animals naturally infected with a field strain of a PRRS virus and porcine animals vaccinated with the vaccines of the invention, which vaccines (viruses) preferably evidence a decreased interferon- α inhibitory effect as compared to wild-type P129 PRRS virus (SEQ ID NO:5).

BRIEF DESCRIPTION OF THE TABLES AND FIGURES

Table 1 shows infectious cDNA clones and the corresponding viruses that were derived by transfection into PK-9 cells.

Table 2 shows the interferon- α inhibitory effect of wild-type PRRS virus and derivatives adapted to growth in cell culture.

Table 3 delineates the interferon- α inhibitory effect of wild-type PRRS virus P129 and its genetically engineered derivatives adapted to grow in CD163-expressing PK-9 cells.

Table 4 shows decreased interferon- α inhibitory effect of the P129-PK-FL and P129-PK-d43/44 viruses as compared to the wild-type P129 virus and the PRRS Ingelvac vaccines.

Table 5 depicts the design of a study conducted to evaluate the safety and efficacy of vaccine viruses.

Table 6 shows all nucleotide differences and resulting amino acid differences between P129 passage 0 and P129-PK-FL passage 17, by genome position.

Table 7 shows a summary of nucleotide and amino acid differences between P129 passage 0 and P129-PK-FL passage 17, by viral protein.

Table 8 shows all nucleotide differences and resulting amino acid differences between the PRRSV genomes found in infectious cDNA clones pCMV-S-P129 and pCMV-S-P129-PK17-FL, by genome position.

Tables 9 and 10 show amino acid changes contributing to the phenotype of the Passage 52 virus (SEQ ID NO:6).

Table 11 shows numbers of pigs with clinical signs following vaccination at one day of age with a modified live PRRSV vaccine.

Table 12 shows serum mean titers following vaccination at one day of age with a modified live PRRSV vaccine.

Table 13 shows percent lung lesions following a challenge of 7-week old pigs previously vaccinated at one day of age with a modified live PRRSV vaccine.

Table 14 shows percent lung lesions following a challenge of 18-week old pigs previously vaccinated at one day of age with a modified live PRRSV vaccine.

Table 15 shows percent lung lesions following a challenge of 26-week old pigs previously vaccinated at one day of age with a modified live PRRSV vaccine.

Table 16 shows percent lung lesions following a challenge of 5-week old piglets previously vaccinated with a modified live PRRSV vaccine.

FIG. 1 shows rectal temperatures post-vaccination.

FIG. 2 shows rectal temperatures post-challenge with virulent PRRSV NADC20.

FIG. 3 shows body weights post-vaccination and post-challenge.

FIG. 4 shows post-challenge data for percentage of lungs with PRRS lesions.

FIG. 5 shows post-challenge lung assessment scores (LAS) for severity of lesions observed.

FIG. 6 is a histogram that depicts the anti-PRRSV antibody levels in serum post-vaccination and post-challenge (ELISA S/P ratios).

FIG. 7 is a graphical representation of post-challenge virus load in serum (log TCID₅₀/ml on PAM cells)

FIG. 8 is a pictorial representation of the methods employed for obtaining the vaccines including SEQ ID NO:1 through SEQ ID NO:6, as disclosed herein.

BRIEF DESCRIPTION OF THE MAJOR SEQUENCES

SEQ ID NO:1 provides the P129-PK-FL passage 17 complete genome.

SEQ ID NO:2 provides the P129-PK-d43/44 passage 17 complete genome.

SEQ ID NO:3 provides the P129-PK-FL passage 24 complete genome.

SEQ ID NO:4 provides the P129-PK-d43/44 passage 34 complete genome.

SEQ ID NO:5 provides the P129 passage 0 complete genome.

SEQ ID NO:6 provides the P129 passage 52 complete genome.

DETAILED DESCRIPTION OF THE INVENTION

As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, references to “the method” includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described.

The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, e.g., Sambrook, et al. *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); Maniatis et al. *Molecular Cloning: A Laboratory Manual* (1982); DNA Cloning: A Practical Approach, vol. I & II (D. Glover, ed.); Oligonucleotide Synthesis (N. Gait, ed., 1984); Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., 1985); Transcription and Translation (B. Hames & S. Higgins, eds., 1984); Animal Cell Culture (R. Freshney, ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984).

“North American PRRS virus” means any PRRS virus having genetic characteristics associated with a North American PRRS virus isolate, such as, but not limited to the PRRS virus that was first isolated in the U.S. around the early 1990’s (see, e.g., Collins, J. E., et al., 1992, *J. Vet. Diagn. Invest.* 4:117-126); North American PRRS virus isolate MN-1b (Kwang, J. et al., 1994, *J. Vet. Diagn. Invest.* 6:293-296); the Quebec LAF-exp91 strain of PRRS (Mardassi, H. et al., 1995, *Arch. Virol.* 140:1405-1418); and North American PRRS virus isolate VR 2385 (Meng, X.-J et al., 1994, *J. Gen. Virol.* 75:1795-1801). Genetic characteristics refer to genomic nucleotide sequence similarity and amino acid sequence similarity shared by North American PRRS virus strains. Chinese PRRS virus strains generally evidence about 80-93% nucleotide sequence similarity with North American strains.

“European PRRS virus” refers to any strain of PRRS virus having the genetic characteristics associated with the PRRS virus that was first isolated in Europe around 1991 (see, e.g., Wensvoort, G., et al., 1991, *Vet. Q.* 13:121-130). “European PRRS virus” is also sometimes referred to in the art as “Lelystad virus”.

“An effective immunoprotective response”, “immunoprotection”, and like terms, for purposes of the present invention, mean an immune response that is directed against one or more antigenic epitopes of a pathogen so as to protect against infection by the pathogen in a vaccinated animal. For purposes of the present invention, protection against infection by a pathogen includes not only the absolute prevention of infection, but also any detectable reduction in the degree or rate of infection by a pathogen, or any detectable reduction in the severity of the disease or any symptom or condition resulting from infection by the pathogen in the vaccinated animal as compared to an unvaccinated infected animal. An effective immunoprotective response can be induced in animals that have not previously been infected with the pathogen and/or are not infected with the pathogen at the time of vaccination. An effective immunoprotective response can also be induced in an animal already infected with the pathogen at the time of vaccination.

A genetically modified PRRS virus is “attenuated” if it is less virulent than its unmodified parental strain. A strain is “less virulent” if it shows a statistically significant decrease in one or more parameters determining disease severity. Such parameters may include level of viremia, fever, severity of respiratory distress, severity of reproductive symptoms, or number or severity of lung lesions, etc.

“Host cell capable of supporting PRRS virus replication” means a cell which is capable of generating infectious PRRS when infected with a virus of the invention. Such cells include porcine cells of the monocyte/macrophage lineage such as porcine alveolar macrophage cells and derivatives, MA-104 monkey kidney cells and derivatives such as MARC-145 cells, and cells transfected with a receptor for the PRRS virus. The term “host cell capable of supporting PRRS virus replication” may also include cells within a live pig.

“Open reading frame”, or “ORF”, as used herein, means the minimal nucleotide sequence required to encode a particular PRRS virus protein without an intervening stop codon.

“Porcine” and “swine” are used interchangeably herein and refer to any animal that is a member of the family Suidae such as, for example, a pig. The term “PRRS virus”, as used herein, unless otherwise indicated, means any strain of either the North American or European PRRS viruses.

“PRRS” encompasses disease symptoms in swine caused by a PRRS virus infection. Examples of such symptoms include, but are not limited to, fever, abortion in pregnant females, respiratory distress, lung lesions, loss of appetite, and mortality in young pigs. As used herein, a PRRS virus that is “unable to produce PRRS” refers to a virus that can infect a pig, but which does not produce any disease symptoms normally associated with a PRRS infection in the pig.

PRRSV “N protein” or “ORF7” as used herein is defined as a polypeptide that is encoded by ORF7 of both the European and North American genotypes of PRRS virus. Examples of specific isotypes of N protein which are currently known are the 123 amino acid polypeptide of the North American PRRS prototype isolate VR2322 reported in Genbank by Accession numbers PRU87392, and the 128 residue N protein of European prototype PRRS isolate Lelystad reported in Genbank Accession number A26843.

“PRRSV N protein NLS-1 region” or “PRRSV ORF7 NLS-1 region” refers to a “pat4” or “nuc1” nuclear localization signal (Nakai & Kanehisa, 1992; Rowland & Yoo, 2003) containing four continuous basic amino acids (lysine or arginine), or three basic residues and a histidine or proline, located within about the first 15 N-terminal residues of the mature N protein. By way of example the VR2332 NLS-1 region sequence is KRKK and is located at residues 9-12, while the Lelystad isolate sequence is KKKK and is located at residues 10-13 of the N protein.

“PRRSV N protein NLS-2 region” or “PRRSV ORF7 NLS-2 region” refers to a second nuclear localization signal within the N protein that can take one of two forms. In North American PRRS viruses NLS-2 has a pattern which we have designated as the “pat8” motif, which begins with a proline followed within three residues by a five residue sequence containing at least three basic residues (K or R) out of five (a slight modification of the “pat7” or “nuc2” motif described by Nakai & Kanehisa, 1992; Rowland & Yoo, 2003). By way of example such a sequence is located at N protein residues 41-47 of the North American PRRSV isolate VR2332, and is represented by the sequence P . . . K. In European PRRS viruses NLS-2 has a “pat4” or “nuc1” motif, which is a continuous stretch of four basic amino acids or three basic residues associated with histidine or proline (Nakai & Kanehisa, 1992; Rowland & Yoo, 2003). The NLS-2 of the European PRRSV isolate Lelystad is located at residues 47-50 and is represented by the sequence K . . . K.

“PRRSV N protein NoLS region” or “PRRSV ORF7 NoLS region” refers to a nucleolar localization signal having a total length of about 32 amino acids and incorporating the NLS-2 region near its amino terminus. By way of example the VR2332 NoLS region sequence is located at residues 41-72 and is represented by the sequence P . . . R (Rowland & Yoo, 2003) and the corresponding Lelystad isolate sequence is located at residues 42-73 and is represented by the sequence P . . . R.

“Transfected host cell” means practically any host cell which as described in U.S. Pat. No. 5,600,662 when transfected with PRRS virus RNA can produce at least a first round of PRRS virions.

An “infectious DNA molecule”, for purposes of the present invention, is a DNA molecule that encodes the necessary elements to support replication, transcription, and translation into a functional virion from a suitable host cell.

Likewise, an “isolated polynucleotide molecule” refers to a composition of matter comprising a polynucleotide mol-

ecule of the present invention purified to any detectable degree from its naturally occurring state, if any.

For purposes of the present invention, the nucleotide sequence of a second polynucleotide molecule (either RNA or DNA) is “homologous” to the nucleotide sequence of a first polynucleotide molecule, or has “identity” to said first polynucleotide molecule, where the nucleotide sequence of the second polynucleotide molecule encodes the same polyaminoacid as the nucleotide sequence of the first polynucleotide molecule as based on the degeneracy of the genetic code, or when it encodes a polyaminoacid that is sufficiently similar to the polyaminoacid encoded by the nucleotide sequence of the first polynucleotide molecule so as to be useful in practicing the present invention. Homologous polynucleotide sequences also refers to sense and anti-sense strands, and in all cases to the complement of any such strands. For purposes of the present invention, a polynucleotide molecule is useful in practicing the present invention, and is therefore homologous or has identity, where it can be used as a diagnostic probe to detect the presence of PRRS virus or viral polynucleotide in a fluid or tissue sample of an infected pig, e.g. by standard hybridization or amplification techniques. Generally, the nucleotide sequence of a second polynucleotide molecule is homologous to the nucleotide sequence of a first polynucleotide molecule if it has at least about 70% nucleotide sequence identity to the nucleotide sequence of the first polynucleotide molecule as based on the BLASTN algorithm (National Center for Biotechnology Information, otherwise known as NCBI, (Bethesda, Md., USA) of the United States National Institute of Health). In a specific example for calculations according to the practice of the present invention, reference is made to BLASTP 2.2.6 [Tatusova TA and TL Madden, “BLAST 2 sequences—a new tool for comparing protein and nucleotide sequences.” (1999) FEMS Microbiol Lett. 174:247-250.]. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 0.1, and the “blosum62” scoring matrix of Henikoff and Henikoff (Proc. Nat. Acad. Sci. USA 89:10915-10919, 1992). The percent identity is then calculated as: Total number of identical matches×100/divided by the length of the longer sequence+number of gaps introduced into the longer sequence to align the two sequences.

Preferably, a homologous nucleotide sequence has at least about 75% nucleotide sequence identity, even more preferably at least about 80%, 85%, 90%, 95%, 96%, 97%, 98% and 99% nucleotide sequence identity. Since the genetic code is degenerate, a homologous nucleotide sequence can include any number of “silent” base changes, i.e. nucleotide substitutions that nonetheless encode the same amino acid.

A homologous nucleotide sequence can further contain non-silent mutations, i.e. base substitutions, deletions, or additions resulting in amino acid differences in the encoded polyaminoacid, so long as the sequence remains at least about 70% identical to the polyaminoacid encoded by the first nucleotide sequence or otherwise is useful for practicing the present invention. In this regard, certain conservative amino acid substitutions may be made which are generally recognized not to inactivate overall protein function: such as in regard of positively charged amino acids (and vice versa), lysine, arginine and histidine; in regard of negatively charged amino acids (and vice versa), aspartic acid and glutamic acid; and in regard of certain groups of neutrally charged amino acids (and in all cases, also vice versa), (1) alanine and serine, (2) asparagine, glutamine, and histidine, (3) cysteine and serine, (4) glycine and proline, (5) isoleu-

cine, leucine and valine, (6) methionine, leucine and isoleucine, (7) phenylalanine, methionine, leucine, and tyrosine, (8) serine and threonine, (9) tryptophan and tyrosine, (10) and for example tyrosine, tryptophan and phenylalanine. Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions may be found in WO 97/09433, page 10, published Mar. 13, 1997 (PCT/GB96/02197, filed Sep. 6, 1996). Alternatively, conservative amino acids can be grouped as described in Lehninger, (Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp. 71-77). Additional suitable conservative changes and the application thereof are described below.

Homologous nucleotide sequences can be determined by comparison of nucleotide sequences, for example by using BLASTN, above. Alternatively, homologous nucleotide sequences can be determined by hybridization under selected conditions. For example, the nucleotide sequence of a second polynucleotide molecule is homologous to SEQ ID NO:1 (or any other particular polynucleotide sequence) if it hybridizes to the complement of SEQ ID NO:1 under moderately stringent conditions, e.g., hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65° C., and washing in 0.2×SSC/0.1% SDS at 42° C. (see Ausubel et al editors, Protocols in Molecular Biology, Wiley and Sons, 1994, pp. 6.0.3 to 6.4.10), or conditions which will otherwise result in hybridization of sequences that encode a PRRS virus as defined below. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, et al., (Eds.), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y. (1989), pp. 9.47 to 9.51.

In another embodiment, a second nucleotide sequence is homologous to SEQ ID NO:1 (or any other sequence of the invention) if it hybridizes to the complement of SEQ ID NO:1 under highly stringent conditions, e.g. hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% SDS, 1 mM EDTA at 65° C., and washing in 0.1×SSC/0.1% SDS at 68° C., as is known in the art (Ausubel et al. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York, 1989).

It is furthermore to be understood that the isolated polynucleotide molecules and the isolated RNA molecules of the present invention include both synthetic molecules and molecules obtained through recombinant techniques, such as by in vitro cloning and transcription.

Polynucleotide molecules can be genetically mutated using recombinant techniques known to those of ordinary skill in the art, including by site-directed mutagenesis, or by random mutagenesis such as by exposure to chemical mutagens or to radiation, as known in the art." The mutations may be carried out by standard methods known in the art, e.g. site directed mutagenesis (see e.g. Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.) of an infectious copy as described (e.g. Meulenberg et al., *Adv. Exp. Med. Biol.*, 1998, 440:199-206).

Accordingly, the subject invention further provides a method for making a genetically modified North American PRRS virus, which method comprises mutating the DNA

sequence encoding an infectious RNA molecule which encodes the PRRS virus as described above, and expressing the genetically modified PRRS virus using a suitable expression system. A genetically modified PRRS virus can be expressed from an isolated polynucleotide molecule using suitable expression systems generally known in the art, examples of which are described in this application. For example, the isolated polynucleotide molecule can be in the form of a plasmid capable of expressing the encoded virus in a suitable host cell in vitro, as is described in further detail below.

The North American PRRSV N protein sequences are highly conserved and the reported sequences have about 93-100% identity with each other. The North American and European PRRSV N proteins are about 57-59% identical and share common structural motifs. Generally, when comparing PRRS encoding sequences and isolates, which might be numbered differently as to specific nucleotides or encoded amino acids, identification of the proper regions are readily achieved by identifying preserved characteristic amino acids in a PRRS strain of interest and aligning it with a reference strain.

Recombinant DNA technology comprises extremely varied and powerful molecular biology techniques aimed at modifying nucleic acids at the DNA level and makes it possible to analyze and modify genomes at the molecular level. In this respect, viruses such as the PRRS virus because of the modest size of its genome is particularly amenable to such manipulations. However, recombinant DNA technology is not immediately applicable to non-retroviral RNA viruses because these viruses do not encompass a DNA intermediate step in their replication. For such viruses, infectious cDNA clones have to be developed before recombinant DNA technology can be applied to their genome to generate modified virus. Infectious clones can be derived through the construction of full-length (genomic length) cDNA (here used in the broad sense of a DNA copy of RNA and not only in the strict sense of a DNA copy of mRNA) of the virus under study, after which an infectious transcript is synthesized in vivo in cells transfected with the full-length cDNA, but infectious transcripts can also be obtained by in vitro transcription from full-length cDNA in a plasmid having a prokaryotic promoter in the presence of a transcription cocktail, or again in vitro using ligated partial-length cDNA fragments that comprise the full viral genome. In all cases, the transcribed RNA carries all the modifications that have been introduced to the cDNA and can be used to further passage the thus modified virus.

The preparation of an infectious clone of a European PRRS virus isolate or Lelystad virus is described in U.S. Pat. No. 6,268,199 which is hereby fully incorporated by reference. The preparation of an infectious cDNA clone of a North American PRRS virus isolate designated P129 (Lee et al., 2005; Yoo et al., 2004) is described in U.S. Pat. No. 6,500,662 which is hereby incorporated fully by reference. The sequence of P129 cDNA is disclosed in Genbank Accession Number AF494042 and in U.S. Pat. No. 6,500,662. Our work below makes use of such an infectious clone which in the context of a plasmid is expressed by the CMV immediate early promoter and has been designated pCMV-S-P129 and is also disclosed within U.S. Pat. No. 6,500,662. As described in U.S. Pat. No. 6,500,662 there are other plasmids and promoters suitable for use here.

Given the complete sequence of any open reading frame of interest and the location of an amino acid residue of interest, one of ordinary skill need merely consult a codon table to design changes at the particular position desired.

Codons constitute triplet sequences of nucleotides in mRNA and their corresponding cDNA molecules. Codons are characterized by the base uracil (U) when present in a mRNA molecule but are characterized by base thymidine (T) when present in DNA. A simple change in a codon for the same amino acid residue within a polynucleotide will not change the sequence or structure of the encoded polypeptide. It is apparent that when a phrase stating that a particular 3 nucleotide sequence "encode(s)" any particular amino acid, the ordinarily skilled artisan would recognize that the table above provides a means of identifying the particular nucleotides at issue. By way of example, if a particular three nucleotide sequence encodes lysine, the table above discloses that the two possible triplet sequences are AAA and AAG. Glycine is encoded by GGA, GGC, GGT (GGU if in RNA) and GGG. To change a lysine to glycine residue in an encoded protein one might replace a AAA or AAG triplet with any of by GGA and GGC, GGT or GGG in the encoding nucleic acid.

As aforementioned, the present invention is directed to the provision of vaccine strains of PRRS wherein host responses to the virus that are mediated by interferon pathways, among other responses, are not downregulated. As described in detail below, there are various modifications to the viral genome that are effective in this regard, particularly those found in ORF1a as disclosed herein, and combinations thereof. It should be noted that similar modification points can be found in additional open reading frames of the PRRS genome, as also disclosed herein (see Table 9).

It is noteworthy that certain other prior approaches to modification of the PRRS polynucleotide have been successful in order to attenuate the PRRS virus, possibly providing suitability for vaccine use, although the exact cause of the resultant attenuations is not generally known. For example, it has been disclosed to attenuate a virulent PRRS virus by mutating or deleting the NLS-2 region, NoLS region, or the NES region in the nucleocapsid or N protein (encoded by ORF7) of the virus, to include a deletion in open reading frame 7 (ORF7). In another aspect the ORF7 deletion is within the sequence encoding a nuclear localization signal (NLS) of a capsid protein. The ORF7 deletion within the sequence encoding an NLS may include deletion of one or more amino acids at positions 43-48 or deletion of an amino acid at either or both positions 43 and 44. See, for example, the entire disclosure of U.S. Pat. No. 7,544,362 which is incorporated by reference. The nucleocapsid protein (N) of PRRSV, which is encoded by ORF7, is a small basic protein that is phosphorylated (Wootton, Rowland, and Yoo, 2002) and forms homodimers (Wootton and Yoo, 2003). The crystal structure has recently been determined (Doan and Dokland, 2003). The N protein appears to have multiple functions in the infected cell. In addition to forming a spherical capsid structure into which genomic RNA is packaged, a process that takes place in the cytoplasm, a portion of N protein is transported into the nucleus and specifically to the nucleolus of the infected cell. The amino acid sequence of N protein contains two nuclear localization signals (NLS), a nucleolar localization signal (NoLS), and a nuclear export signal (NES) that facilitate transport into the nucleus and nucleolus, and export from the nucleus, respectively (Rowland et al., 1999; Rowland et al., 2003; Rowland and Yoo, 2003). While in the nucleolus, the N protein interacts with the small nucleolar RNA-associated protein fibrillarin and may regulate rRNA processing and ribosome biogenesis in the infected cell in order to favor virus replication (Yoo et al., 2003). Viral mutations of this type are valuable, either alone or in combination with other attenu-

ating mutations, for designing novel PRRS vaccines. In another example of a PRRS virus that has been attenuated, modified to ORF1a was employed. Deletion of the DNA sequence encoding the antigenic epitope between amino acids 616 to 752 in the hypervariable region in the non-structural protein 2 coding region of ORF1a was employed, see. U.S. Pat. No. 7,618,797, which is incorporated by reference in its entirety.

Studies on the immunobiology of PRRS virus are suggestive that the interaction of PRRS virus with PDCs merits examination. This cell type represents 0.2%-0.8% of peripheral blood mononuclear cells in humans, mice, rats, pigs and monkeys. Despite its scarcity, this cell is an important component of the innate immune system and is capable of secreting copious amounts of IFN- α following viral stimulation. It is through the secretion of IFN- α that PDCs play a major role in regulating antiviral innate and adaptive immunity since they promote the function of natural killer cells, B cells, and T cells. Furthermore, the maturation of porcine monocyte derived dendritic cells (MoDC) is aided by the IFN- α secreted by PDCs resulting in an enhanced ability of MoDCs to present antigen and activate T cells. At a later stage of viral infection, PDCs differentiate into a unique type of mature dendritic cell, which directly regulates the function of T cells and direct the differentiation of T cells into cells capable of secreting IFN- γ , which is a major mediator of antiviral immunity against viruses including PRRS virus. Not surprisingly there are human viruses, such as respiratory syncytial virus and measles virus, which are known to suppress the ability of PDCs to secrete IFN- α . This inhibitory effect is thought to play a role in the predominance of a humoral immune response and the associated immunopathology observed as a result of the infection with these viruses, as well as in the increased susceptibility of the host to secondary bacterial and viral infections.

In contrast, the wild-type PRRSV isolates as well as both of the Ingelvac PRRS vaccine strains (see Examples 5 and following, below) inhibited the ability of purified populations of porcine PDC to produce IFN- α , while the novel P129-PK-FL and P129-PK-d43/44 virus stocks (see below) exhibited a minimal to nil inhibitory effect on this PDC function. The significance of these observations resides, in part, on the importance of IFN- α in regulating the development of the adaptive immune response to viruses. Accordingly, it is very likely that an attenuated virus vaccine derived from a minimally IFN- α suppressing virus would elicit a strong antiviral protective immune response. It has previously been demonstrated the adjuvant effect of IFN- α on the Ingelvac PRRS MLV vaccine induced virus-specific T cell mediated IFN- γ response, and that the intensity of the virus-specific T cell mediated IFN- γ response elicited by the vaccine has a positive correlation with protective immunity against the virus under field and laboratory conditions. Accordingly, although not being limited as to theory, it would be reasonable to expect that the cell-mediated immune response and level of protective immunity elicited by a non-IFN- α -inhibitory PRRSV will be significantly greater than that of a PRRSV isolate exhibiting the wild-type (IFN- α inhibitory) phenotype.

Referring to the present invention, it is notable that the P129-PK-FL virus as well as all five deletion mutants derived from the pCMV-S-P129-PK infectious cDNA clone lost the ability to inhibit IFN- α production. Therefore this unusual phenotype can not be solely due to the deletions, but must be due at least in part to genetic changes that became fixed during construction of the infectious clone. Interestingly, uncloned P129 virus that was serially passaged 63

times on PK-9 cells retained the ability to inhibit IFN induction (Table 1). The most likely explanation for the common IFN phenotype seen in all infectious clone-derived viruses is the incorporation of one or more mutations during the generation of the infectious clone. These mutations would have existed, possibly at low levels, in the viral RNA used to construct the infectious clone. Ultimately, the mutations may have existed in the original (passage 0) virus in the pig or they may have been generated and enriched during the process of adapting the virus to growth on PK-9 cells for 16 passages. The possibility that the mutations were the result of PCR-induced errors or cloning artifacts cannot be ruled out. At any rate, the mutation(s) responsible for the loss of the IFN- α inhibitory function became "fixed" during infectious clone construction, and would be expected to be present in all viruses derived from this particular infectious clone.

The possibility that mutations responsible for the altered IFN- α inhibition phenotype pre-existed in the viral RNA used to construct the cDNA clone is likely, given that PRRSV is known to readily generate random genetic diversity as a result of errors by the viral RNA-dependent RNA polymerase. Virus quasi-species are comprised of a heterogeneous mixture of closely related genetic variants that naturally appear during virus replication in vivo. Even more relevant is the observation of virus quasi-species after multiple in vitro passages of PRRSV derived from an infectious cDNA clone. This is notable since the starting population of virus genomes in previously conducted studies consisted of a genetically homogenous population, and sequence diversity was rapidly generated during passage in cell culture. In the current study, the level of genomic heterogeneity would have been higher, since the original P129 virus had not been cloned (biologically or molecularly) prior to the 16 PK-9 passages leading up to construction of the infectious clone. Thus the chance selection of a PRRSV RNA variant responsible for the loss of IFN- α inhibition function from among the quasi-species, and incorporation into the pCMV-S-P129-PK17-FL infectious cDNA clone seems plausible. The incorporation of these mutations into an infectious clone might be considered fortuitous, under some circumstances, given that all derivative viruses should share this distinct biological phenotype which may prove important for the development of effective next-generation PRRS vaccines.

The wild-type PRRS virus strain P129, like other strains of this virus, exhibited a strong inhibitory effect on the ability of peripheral blood mononuclear cells (PBMCs) and plasmacytoid dendritic cells (PDCs) to produce interferon (IFN)- α . On the other hand, virus derived from an infectious cDNA clone of P129 (pCMV-S-P129-PK17-FL) exhibited a significant reduction in the IFN- α inhibitory phenotype. This infectious clone was constructed from virus which was previously adapted to grow on the CD163-expressing porcine kidney cell line PK-9 over the course of 16 serial passages (see U.S. Pat. No. 7,754,464 which is incorporated by reference in its entirety). The IFN- α inhibitory phenotype of P129-PK-FL and P129-PK-d43/44 ranged from low to negligible and was in marked contrast to that exhibited by either of the two Ingelvac PRRS modified live virus vaccine strains, both of which were highly inhibitory. These results indicate that the P129-PK-FL and P129-PK-d43/44 viruses are biologically distinct from the parental low-passage P129 isolate, other wild-type PRRS viruses, and both Ingelvac PRRS vaccines. The potential implications of the reduced IFN- α -inhibitory phenotype, as well as possible reasons for the phenotypic change, are discussed.

Amino Acid Modifications of the Invention

According to the practice of the present invention, novel isolates of PRRS, whether of North American or Chinese genotypes, may be field-identified that contain specific amino acids at specific positions in the proteins encoded from ORF1, and which confer desirable phenotypes on these viruses. In the alternative, as aforementioned, standard genetic procedures may be employed to modify the genetic sequence (and thus the amino acid sequence) of the encoded ORF1 protein, again to produce modified North American and Chinese PRRS viruses, and infectious clones, and vaccines therefrom, all which provide such phenotypes. In preferred examples the phenotypes include, without limitation, decreased interferon- α inhibitory effect as compared to wild-type PRRS virus, and, optionally, the ability to reproduce or persist in a host animal (a pig) while triggering a robust immune response, but with little detectable pathology.

Thus, in the practice of the invention, North American PRRS strains or isolates that may serve as useful starting points include those disclosed, for example in U.S. Pat. Nos. 6,500,662; 7,618,797; 7,691,389, 7,132,106; 6,773, 908; 7,264,957; 5,695,766; 5,476,778; 5,846,805; 6,042,830; 6,982,160; 6,241,990; and 6,110,468. In regard of Chinese PRRS strains and isolates that may serve as useful starting points, see for example, published Chinese application CN200910091233.6 from Chinese application CN201633909 pertaining to the TJM-92 virus.

In connection with the discussion that follows, internationally recognized single and three-letter designations are used for the most common amino acids encoded by DNA: alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N); aspartic acid (Asp, D); cysteine (Cys, C); glutamic acid (Glu, E); glutamine (Gln, Q); glycine (Gly, G); histidine (His, H); isoleucine (Ile, I); leucine (Leu, L); lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); proline (Pro, P); serine (Ser, S); threonine (Thr, T); tryptophan (Trp, W); tyrosine (Tyr, Y) and Valine (Val, V).

Tables 9 and 10 identify observed amino acid changes responsible for attenuation of virulence in North American (and Chinese) PRRS which correlate with reduced inhibition of interferon alpha activity, thereby permitting a safe and robust immune response to vaccines. Table 10 identifies highly preferred amino acid modifications in this regard, within ORF1a, and shows how these mutations have arisen in regard of passaging from other P129 cultures (it should be noted that the inspection of this history also facilitates design of mutagenesis strategies to (re) construct encoding DNA having any of the amino acid changes of the invention, as needed). In this regard, the most preferred amino acid improvements to ORF1a (as evidenced by P129 passage 52 include: asparagine at 182, asparagine at 189, tyrosine at 273, histidine at 302, threonine at 665, cysteine at 943, threonine at 1429, alanine at 1505, asparagine at 2410, which also potentially adds numerous glycosylation opportunities and which may further alter protein function.

Accordingly, the invention provides an isolated Porcine Reproductive and Respiratory Syndrome Virus (PRRS) wherein the protein thereof encoded by ORF1a is selected from a group consisting of those amino acid sequences that contain any of:

- an amino acid N within the amino acid sequence AMA NVYD (SEQ ID NO: 9);
- an amino acid N within the amino acid sequence IGHNAVM (SEQ ID NO: 12);
- an amino acid D within the amino acid sequence TVPDGNC (SEQ ID NO: 15);

an amino acid Y within the amino acid sequence CWWYLFD (SEQ ID NO: 18);
 an amino acid H within the amino acid sequence HGVHGKY (SEQ ID NO: 21);
 an amino acid V within the amino acid sequence AAK VDQY (SEQ ID NO: 24);
 an amino acid T within the amino acid sequence PSATTDTS (SEQ ID NO: 27);
 an amino acid L within the amino acid sequence LNSLLSK (SEQ ID NO: 30).
 an amino acid C within the amino acid sequence APM CQDE (SEQ ID NO: 33);
 an amino acid T within the amino acid sequence CAPTGMD (SEQ ID NO: 36);
 an amino acid A within the amino acid sequence PKVAKVS (SEQ ID NO: 39);
 an amino acid I within the amino acid sequence AGEIVG (SEQ ID NO: 42);
 an amino acid N within the amino acid sequence ADFNNPEK (SEQ ID NO: 45); and
 an amino acid I within the amino acid sequence QTPILGR (SEQ ID NO: 48).

More specifically, the invention provides an isolated Porcine Reproductive and Respiratory Syndrome Virus (PRRS) wherein the protein thereof encoded by ORF1a is selected from a group consisting of those amino acid sequences that contain any of:

an amino acid N within the amino acid sequence ANV (see SEQ ID NO: 9);
 an amino acid N within the amino acid sequence HNA (see SEQ ID NO: 12);
 an amino acid D within the amino acid sequence PDG (see SEQ ID NO: 15);
 an amino acid Y within the amino acid sequence WYL (see SEQ ID NO: 18);
 an amino acid H within the amino acid sequence VHG (see SEQ ID NO: 21);
 an amino acid V within the amino acid sequence KVD (see SEQ ID NO: 24);
 an amino acid T within the amino acid sequence ATD (see SEQ ID NO: 27);
 an amino acid L within the amino acid sequence SLL (see SEQ ID NO: 30).
 an amino acid C within the amino acid sequence MCQ (see SEQ ID NO: 33);
 an amino acid T within the amino acid sequence PTG (see SEQ ID NO: 36);
 an amino acid A within the amino acid sequence VAK (see SEQ ID NO: 39);
 an amino acid I within the amino acid sequence EIV (see SEQ ID NO: 42);
 an amino acid N within the amino acid sequence FNP (see SEQ ID NO: 45); and
 amino acid I within the amino acid sequence PIL (see SEQ ID NO: 48).

As aforementioned, there are numerous known strains and isolates of North American and Chinese PRRS, and novel strains continue to evolve or to be isolated. Although a high level of amino acid sequence homology exists between all these strains, those skilled in the art will immediately recognize that some variation does exist, and indeed advantage can be taken of these differences and similarities to further improve the phenotypic properties of all vaccine strains.

First, in regard of all of the amino acid motifs defined by SEQ ID NOS as specified directly (on Pages 27-28) above, the underlined and preferred amino acids (as provided from

P129 passage 52) generally remain fully beneficial even if adjacent amino acids have otherwise changed from the specified SEQ ID NO sequences. Thus in regard of AMA NVYD (SEQ ID NO: 9), as a specific and representative example, it is generally possible to inspect the corresponding ORF1-expressed protein sequence from any North American or Chinese PRRS, to find the corresponding amino acid motif, even if additional changes have occurred in such other strains, as a result of evolution, causing substitutions and/or deletions or additions. As will be appreciated by those skilled in the art, the preferred amino acid changes evidenced by P129 passage 52 should thus also remain operable in spite of other changes in overall amino acid sequence that are directly 5' or 3' to the specified amino acid of Passage 52. This will be so especially if the comparative amino acid changes are considered conservative. Thus in regard of AMANVYD (SEQ ID NO: 9), and the subsequence ANV thereof, it should be readily possible to identify the comparable motif in another PRRS strain if, for example, the valine therein is replaced by isoleucine or leucine, or any other residue, or if a residue is simply missing or an additional residue added. Numerous computer programs exist to identify alignments and thus determine if polypeptide sequence motifs correspond, for example the so-called Blosom tables (based on a given level of percent identity), see S. Henikoff et al. "Amino Acid Substitution matrices from protein blocks", Proc Natl Acad Sci, USA, 89(22), pp. 10915-10919, Nov. 15, 1992, 925 and see also A. L. Lehninger et al. Principles of Biochemistry, 2005, MacMillan and Company, 4th edition. Conservative amino acid changes are also recognized based on categorization into 5 overall groups: sulfydryl (Cys); aromatic (Phe, Tyr, and Trp); basic (Lys, Arg, His); aliphatic (Val, Ileu, Leu, Met), and hydrophilic (Ala, Pro, Gly, Glu, Asp, Gln, Asn, Ser and Thr). Thus it is within the practice of the invention to modify any North American or Chinese PRRS encoding nucleotide sequence to incorporate at the appropriate and corresponding position, any of the amino acid changes specified for P129 passage 52, even if one or more of the other amino acids adjacent to the designated position have been added, deleted or substituted.

Additionally, based on similar principles, those skilled in the art will recognize that once a preferred amino acid is identified from the specific Passage 52 changes identified for ORF1a according to the practice of the present invention, that conservative replacements for any such passage 52 amino acids can then also be used, either in P129 variants, or in regard of any other North American or Chinese strains, with substantial preservation of the intended passage 52 phenotype. Thus, as representative examples: in regard of S LL (within SEQ ID NO: 30), the designated leucine residue may be further replaced with isoleucine, valine or methionine; in regard of FNP (within SEQ ID NO: 45), the designated asparagine may be replaced with any of Ala, Pro, Gly, Glu, Asp, Gln, Ser and Thr; and in regard of VAK (see SEQ ID NO: 39), the designated alanine may be replaced with any of Asn, Pro, Gly, Glu, Asp, Gln, Ser and Thr; all and the like, although it will be readily recognized that it is not a requirement of the present invention that any such replacement amino acids work as well as originally identified unique Passage 52 amino acid changes, at the specified locations. Of course, use of standard conservative amino acid changes according to any other recognized model also is practiced in the present invention. For example, and including vice-versa in all cases, Asp for Glu and vice versa, Asn for Gln, Arg for Lys, Ser for Cys or Thr, Phe for Tyr, Val for Leu or Ileu, Ala for Gly, and the like.

Further, within the practice of the present invention, although any of the individual Passage 52 amino acid changes (as identified for ORF1a above) can be usefully placed in any North American or Chinese PRRS with desired phenotypic effect, it is further preferred to include as many of the Table 9 or Table 10 amino acid selections as possible in a final construct, as typically provided for by appropriate modification of the encoding polynucleotide sequence. Thus, the practice of the present invention includes the provision of Chinese or North American PRRS viruses (and corresponding encoding polynucleotides) that provide for 2, 3, 4, 5, 6, 7, 8, 9, and up to any of the approximately 17 identified Passage 52 ORF1a changes, (Table 9) all within a final viral sequence, to include any specific pairs, triplets, or other higher combinations of all the total identified Passage 52 amino acid changes. Such amino acid changes may, of course, be introduced into the corresponding encoding nucleotide sequences of the virus by site directed mutagenesis, PCR, and other techniques as are well known in the art.

To demonstrate that a particular genetically modified strain is attenuated an experiment described as follows may be used.

At least 10 gilts per group are included in each trial, which are derived from a PRRSV-free farm. Animals are tested free of PRRS virus specific serum antibodies and negative for PRRSV. All animals included in the trial are of the same source and breed. The allocation of the animals to the groups is randomized.

Challenge is performed at day 90 of pregnancy with intranasal application of 1 ml PRRSV with 10^5 TCID₅₀ per nostril. There are at least three groups for each test setup: One group for P129 challenge; one test group for challenge with the possibly attenuated virus; and one strict control group.

The study is deemed valid when the strict controls stay PRRSV-negative over the time course of the study and at least 25% less live healthy piglets are born in the P129 challenged group compared to the strict controls.

Attenuation, in other words less virulence, is defined as the statistical significant change of one or more parameters determining reproductive performance or other symptomology:

Significant reduction in at least one of the following parameters for the test group (possibly attenuated virus) compared to the unmodified parental strain infected group would be an indication of attenuation:

- a) frequency of stillborns
- b) abortion at or before day 112 of pregnancy
- c) number of mummified piglets
- d) number of less lively and weak piglets
- e) pre-weaning mortality

Furthermore a significant increase in one of the following parameters for the test group compared the unmodified parental strain infected group is preferred:

- f) number of piglets weaned per sow
- g) number of live healthy piglets born per sow

In the alternative, respiratory symptoms and other symptoms of PRRSV infection could be examined to establish attenuation.

An attenuated strain is valuable for the formulation of vaccines. The present vaccine is effective if it protects a pig against infection by a PRRS virus. A vaccine protects a pig against infection by a PRRS virus if, after administration of the vaccine to one or more unaffected pigs, a subsequent challenge with a biologically pure virus isolate (e.g., VR 2385, VR 2386, P129 etc.) results in a lessened severity of

any gross or histopathological changes (e.g., lesions in the lung) and/or of symptoms of the disease, as compared to those changes or symptoms typically caused by the isolate in similar pigs which are unprotected (i.e., relative to an appropriate control). More particularly, the present vaccine may be shown to be effective by administering the vaccine to one or more suitable pigs in need thereof, then after an appropriate length of time (e.g., 4 weeks), challenging with a large sample ($10^{(3-7)}$ TCID₍₅₀₎) of a biologically pure PRRSV isolate. A blood sample is then drawn from the challenged pig after about one week, and an attempt to isolate the virus from the blood sample is then performed. Isolation of a large amount of the virus is an indication that the vaccine may not be effective, while isolation of reduced amounts of the virus (or no virus) is an indication that the vaccine may be effective.

Thus, the effectiveness of the present vaccine may be evaluated quantitatively (i.e., a decrease in the percentage of consolidated lung tissue as compared to an appropriate control group) or qualitatively (e.g., isolation of PRRSV from blood, detection of PRRSV antigen in a lung, tonsil or lymph node tissue sample by an immunoassay). The symptoms of the porcine reproductive and respiratory disease may be evaluated quantitatively (e.g., temperature/fever) or semi-quantitatively (e.g., the presence or absence of one or more symptoms or a reduction in severity of one or more symptoms, such as cyanosis, pneumonia, lung lesions etc.).

An unaffected pig is a pig which has either not been exposed to a porcine reproductive and respiratory disease infectious agent, or which has been exposed to a porcine reproductive and respiratory disease infectious agent but is not showing symptoms of the disease. An affected pig is one which shows symptoms of PRRS or from which PRRSV can be isolated.

Vaccines of the present invention can be formulated following accepted convention to include acceptable carriers for animals, including humans (if applicable), such as standard buffers, stabilizers, diluents, preservatives, and/or solubilizers, and can also be formulated to facilitate sustained release. Diluents include water, saline, dextrose, ethanol, glycerol, and the like. Additives for isotonicity include sodium chloride, dextrose, mannitol, sorbitol, and lactose, among others. Stabilizers include albumin, among others. Other suitable vaccine vehicles and additives, including those that are particularly useful in formulating modified live vaccines, are known or will be apparent to those skilled in the art. See, e.g., Remington's Pharmaceutical Science, 18th ed., 1990, Mack Publishing, which is incorporated herein by reference.

Vaccines of the present invention can further comprise one or more additional immunomodulatory components such as, e.g., an adjuvant or cytokine, among others. Non-limiting examples of adjuvants that can be used in the vaccine of the present invention include the RIBI adjuvant system (Ribi Inc., Hamilton, Mont.), alum, mineral gels such as aluminum hydroxide gel, oil-in-water emulsions, water-in-oil emulsions such as, e.g., Freund's complete and incomplete adjuvants, Block copolymer (CytRx, Atlanta, Ga.), QS-21 (Cambridge Biotech Inc., Cambridge Mass.), SAF-M (Chiron, Emeryville Calif.), AMPHIGEN® adjuvant, saponin, Quil A or other saponin fraction, monophosphoryl lipid A, and Avridine lipid-amine adjuvant. Non-limiting examples of oil-in-water emulsions useful in the vaccine of the invention include modified SEAM62 and SEAM 1/2 formulations. Modified SEAM62 is an oil-in-water emulsion containing 5% (v/v) squalene (Sigma), 1% (v/v) SPAN® 85 detergent (ICI Surfactants), 0.7% (v/v) TWEEN® 80 deter-

gent (ICI Surfactants), 2.5% (v/v) ethanol, 200 pg/ml Quil A, 100 [mgr]g/ml cholesterol, and 0.5% (v/v) lecithin. Modified SEAM 1/2 is an oil-in-water emulsion comprising 5% (v/v) squalene, 1% (v/v) SPAN® 85 detergent, 0.7% (v/v) Tween 80 detergent, 2.5% (v/v) ethanol, 100 .mu.g/ml Quil A, and 50 .mu.g/ml cholesterol. Other immunomodulatory agents that can be included in the vaccine include, e.g., one or more interleukins, interferons, or other known cytokines.

Vaccines of the present invention can optionally be formulated for sustained release of the virus, infectious RNA molecule, plasmid, or viral vector of the present invention. Examples of such sustained release formulations include virus, infectious RNA molecule, plasmid, or viral vector in combination with composites of biocompatible polymers, such as, e.g., poly(lactic acid), poly(lactic-co-glycolic acid), methylcellulose, hyaluronic acid, collagen and the like. The structure, selection and use of degradable polymers in drug delivery vehicles have been reviewed in several publications, including A. Domb et al., 1992, *Polymers for Advanced Technologies* 3: 279-292, which is incorporated herein by reference. Additional guidance in selecting and using polymers in pharmaceutical formulations can be found in texts known in the art, for example M. Chasin and R. Langer (eds), 1990, "Biodegradable Polymers as Drug Delivery Systems" in: *Drugs and the Pharmaceutical Sciences*, Vol. 45, M. Dekker, N.Y., which is also incorporated herein by reference. Alternatively, or additionally, the virus, plasmid, or viral vector can be microencapsulated to improve administration and efficacy. Methods for microencapsulating antigens are well-known in the art, and include techniques described, e.g., in U.S. Pat. Nos. 3,137,631; 3,959,457; 4,205,060; 4,606,940; 4,744,933; 5,132,117; and International Patent Publication WO 95/28227, all of which are incorporated herein by reference.

Liposomes can also be used to provide for the sustained release of virus, plasmid, or viral vector. Details concerning how to make and use liposomal formulations can be found in, among other places, U.S. Pat. Nos. 4,016,100; 4,452,747; 4,921,706; 4,927,637; 4,944,948; 5,008,050; and 5,009,956, all of which are incorporated herein by reference.

An effective amount of any of the above-described vaccines can be determined by conventional means, starting with a low dose of virus, viral protein plasmid or viral vector, and then increasing the dosage while monitoring the effects. An effective amount may be obtained after a single administration of a vaccine or after multiple administrations of a vaccine. Known factors can be taken into consideration when determining an optimal dose per animal. These include the species, size, age and general condition of the animal, the presence of other drugs in the animal, and the like. The actual dosage is preferably chosen after consideration of the results from other animal studies (see, for example, Examples 2 and 3 below).

One method of detecting whether an adequate immune response has been achieved is to determine seroconversion and antibody titer in the animal after vaccination. The timing of vaccination and the number of boosters, if any, will preferably be determined by a doctor or veterinarian based on analysis of all relevant factors, some of which are described above.

The effective dose amount of virus, protein, infectious DNA molecule, plasmid, or viral vector, of the present invention can be determined using known techniques, taking into account factors that can be determined by one of ordinary skill in the art such as the weight of the animal to be vaccinated. The dose amount of virus of the present

invention in a vaccine of the present invention preferably ranges from about 10^1 to about 10^9 pfu (plaque forming units), more preferably from about 10^2 to about 10^8 pfu, and most preferably from about 10^3 to about 10^7 pfu. The dose amount of a plasmid of the present invention in a vaccine of the present invention preferably ranges from about 0.1 mg to about 100 mg, more preferably from about 1 mg to about 10 mg, even more preferably from about 10 mg to about 1 mg. The dose amount of an infectious DNA molecule of the present invention in a vaccine of the present invention preferably ranges from about 0.1 mg to about 100 mg, more preferably from about 1 mg to about 10 mg, even more preferably from about 10 mg to about 1 mg. The dose amount of a viral vector of the present invention in a vaccine of the present invention preferably ranges from about 10^1 pfu to about 10^9 pfu, more preferably from about 10^2 pfu to about 10^8 pfu, and even more preferably from about 10^3 to about 10^7 pfu. A suitable dosage size ranges from about 0.5 ml to about 10 ml, and more preferably from about 1 ml to about 5 ml.

Suitable doses for viral protein or peptide vaccines according to the practice of the present invention range generally from 1 to 50 micrograms per dose, or higher amounts as may be determined by standard methods, with the amount of adjuvant to be determined by recognized methods in regard of each such substance. In a preferred example of the invention relating to vaccination of swine, an optimum age target for the animals is between about 1 and 21 days, which at pre-weaning, may also correspond with other scheduled vaccinations such as against *Mycoplasma hyopneumoniae* or PCV. Additionally, a preferred schedule of vaccination for breeding sows would include similar doses, with an annual revaccination schedule.

An effective amount of any of the above-described vaccines can be determined by conventional means, starting with a low dose of virus, plasmid or viral vector, and then increasing the dosage while monitoring the effects. An effective amount may be obtained after a single administration of a vaccine or after multiple administrations of a vaccine. Known factors can be taken into consideration when determining an optimal dose per animal. These include the species, size, age and general condition of the animal, the presence of other drugs in the animal, and the like. The actual dosage is preferably chosen after consideration of the results from other animal studies.

One method of detecting whether an adequate immune response has been achieved is to determine seroconversion and antibody titer in the animal after vaccination. The timing of vaccination and the number of boosters, if any, will preferably be determined by a doctor or veterinarian based on analysis of all relevant factors, some of which are described above.

The effective dose amount of virus, infectious RNA molecule, plasmid, or viral vector, of the present invention can be determined using known techniques, taking into account factors that can be determined by one of ordinary skill in the art such as the weight of the animal to be vaccinated. By way of example, vaccines may be delivered orally, parenterally, intradermally, subcutaneously, intramuscularly, intranasally or intravenously. Oral delivery may encompass, for example, adding the compositions to the feed or drink of the animals. Factors bearing on the vaccine dosage include, for example, the weight and age of the pig.

The present invention further provides a method of preparing a vaccine comprising a PRRS virus, infectious RNA molecule, plasmid, or viral vector described herein, which method comprises combining an effective amount of one of

25

the PRRS virus, infectious RNA molecule, plasmid, or viral vector of the present invention, with a carrier acceptable for pharmaceutical or veterinary use.

In addition the live attenuated vaccine of the present invention can be modified as described in U.S. Pat. No. 6,500,662 to encode a heterologous antigenic epitope which is inserted into the PRRS viral genome using known recombinant techniques. See also U.S. Pat. No. 7,132,106 which is incorporated by reference in its entirety. Antigenic epitopes useful as heterologous antigenic epitopes for the present invention include antigenic epitopes from a swine pathogen other than PRRS virus which include, but are not limited to, an antigenic epitope from a swine pathogen selected from the group consisting of porcine parvovirus, porcine circovirus, a porcine rotavirus, swine influenza, pseudorabies virus, transmissible gastroenteritis virus, porcine respiratory coronavirus, classical swine fever virus, African swine fever virus, encephalomyocarditis virus, porcine paramyxovirus, torque teno virus, *Actinobacillus pleuropneumoniae*, *Actinobacillus suis*, *Bacillus anthracis*, *Bordetella bronchiseptica*, *Clostridium haemolyticum*, *Clostridium perfringens*, *Clostridium tetani*, *Escherichia coli*, *Erysipelothrix rhusiopathiae*, *Haemophilus parasuis*, *Leptospira* spp., *Mycoplasma hyopneumoniae*, *Mycoplasma hyorhinis*, *Mycoplasma hyosynoviae*, *Pasteurella multocida*, *Salmonella choleraesuis*, *Salmonella typhimurium*, *Streptococcus equis-milis*, and *Streptococcus suis*. Nucleotide sequences encoding antigenic epitopes from the aforementioned swine pathogens are known in the art and can be obtained from public gene databases on the worldwide web, such as at Genbank from the (USA) National Center for Biotechnology Information.

Additional features and variations of the invention will be apparent to those skilled in the art from the entirety of this application, including the detailed description, and all such features are intended as aspects of the invention. Likewise, features of the invention described herein can be re-combined into additional embodiments that also are intended as aspects of the invention, irrespective of whether the combination of features is specifically mentioned above as an aspect or embodiment of the invention. Also, only such limitations which are described herein as critical to the invention should be viewed as such; variations of the invention lacking limitations which have not been described herein as critical are intended as aspects of the invention. It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the invention.

The following examples are intended to illustrate but not limit the invention.

Example 1

Adaptation and Attenuation of PRRSV Isolate P129 to PK-9 Cells

Virulent PRRS isolate P129 was isolated from a sick pig in Indiana in 1995 at the Animal Disease Diagnostic Laboratory of Purdue University. A serum sample from this pig was passaged once in a high health status pig to expand serum and lung homogenate stocks. Viral RNA was extracted from the serum and lung homogenate and used to determine the complete genome consensus sequence of P129 passage 0 virus. RNA was first primed with random

26

hexamers and used to synthesize cDNA. The genome was amplified in three overlapping pieces using high fidelity (proofreading) PCR. The PCR products from three separate PCR reactions (per genome segment) were T/A cloned and used for DNA sequencing to generate a full length genome consensus sequence (see SEQ ID NO:1).

An aliquot of the same pig serum used for DNA sequencing, containing P129 passage 0, was used to infect primary porcine alveolar macrophage (PAM) cells. The progeny virus from the PAM infection (passage 1) was filtered through a 0.1 micrometer syringe filter and used to infect PK-9 cells.

PK-9 cells are a transgenic cell line derived by stably transfecting the PK0809 porcine kidney cell line with a plasmid encoding a deleted version of the porcine CD163 gene and the neomycin resistance gene. Details of the construction and characterization of the PK-9 cell line have been described previously.

Adaptation of the passage 1 virus from PAM cells to growth on PK-9 cells was difficult, and required several attempts with multiple parallel lineages. Infection was monitored by immunofluorescence of duplicate wells using FITC-conjugated monoclonal antibody SDOW17 specific for the viral nucleocapsid protein (Rural Technologies Inc, Brookings S. Dak.). Early passages resulted in a few small foci, but did not generate enough cell-free virus particles to initiate infection of a fresh monolayer. These passages were accomplished by treating the infected monolayer with Accutase (a trypsin substitute) and reseeding the cells in multiple wells with fresh medium, with or without the addition of non-infected PK-9 cells. After several such passages, some lineages showed a clear increase in the frequency and size of fluorescent foci. Some of these had acquired the ability to be passaged using cell-free virus fluids. By passage 17 (1 on PAM cells, 16 on PK-9), one lineage could reliably be sustained using dilutions of the cell-free fluids from the previous passages, and resulted in the infection of the entire monolayer within a few days. The virus did not cause cytopathic effect on PK-9 cells at any passage level. RNA was extracted from infected PK-9 cells at virus passage 17 and used to construct an infectious cDNA clone.

Example 2

Construction of an Infectious cDNA Clone of P129-PK Passage 17

An infectious cDNA clone of the P129-PK passage 17 virus was constructed, using a backbone plasmid as previously described. The genome of the virus was amplified by reverse transcription and PCR in three overlapping segments, with naturally occurring unique restriction endonuclease sites in the regions of overlap. The products from three separate PCR reactions were cloned and sequenced, and aligned to generate a consensus sequence for each genome segment. If none of the three cloned products of a given segment matched the predicted amino acid sequence of the consensus for that segment, one of the clones was modified by subcloning and/or site-directed mutagenesis until it matched the predicted amino acid sequence of the consensus. The three genome segments and the plasmid backbone were joined using standard cloning techniques and restriction endonuclease sites. The resulting full-length clone, designated pCMV-S-P129-PK17-FL, was infectious when transfected into PK-9 cells. The sequence of this infectious cDNA clone is given in SEQ ID NO:2. The

27

genome is essentially identical to the passage 17 virus from which it was constructed, with authentic termini, and lacks any insertions or deletions relative to the consensus sequences of passage 17. There are no engineered restriction sites or other targeted changes within the viral genome of this infectious cDNA clone.

Nucleotide and amino acid differences between the complete genome consensus sequences of P129 passage 0 and the genome sequence of infectious clone pCMV-S-P129-PK17-FL are listed individually in Table 6. Table 6 includes all nucleotide differences and resulting amino acid differences by genome position. A subset of these mutations are responsible for the change in phenotype from IFN inhibitory (passage 0) to IFN non-inhibitory (all viruses derived from the passage 17 infectious cDNA clone). Table 7 summarizes nucleotide, amino acid, and non-conserved amino acid differences by PRRSV open reading frame (ORF) or non-structural protein (nsp). For the purposes of Table 7, the following groups of amino acids are among those considered conserved: [K, R], [D, E], [L, I, V, A], and [S, T].

Example 3

Deletion Mutants in P129-PK Passage 17

Deletions in two areas of the genome were engineered into infectious cDNA clone pCMV-S-P129-PK17-FL, to generate five genetically modified infectious clones.

One area of the genome to undergo modification was the nuclear localization sequence (NLS) located at amino acid positions 41-47 of the nucleocapsid protein (encoded by PRRSV ORF 7). Two types of deletions were made. These deletions have been described previously within the context of another PRRSV infectious clone. The wild type sequence of amino acid residues 41-49 is PG . . . KN. In mutant "d43/44", also known as "PG-KS", lysine residues 43 and 44 are deleted and asparagine residue 49 is changed to a serine. In mutant "d43144146", also known as "PG-S-KS", lysine residues 43, 44, and 46 are deleted and asparagine residue 49 is changed to a serine. The infectious clones derived from pCMV-S-P129-PK17-FL that incorporate these deletions are pCMV-S-P129-PK17-d43/44 and pCMV-S-P129-PK17-d43/44/46 respectively. See U.S. Pat. No. 7,544,362.

The second area of the genome to undergo modification was in the hypervariable region of nsp2, within ORF1a. A deletion of 131 amino acids (393 nucleotides) has been described previously within the context of another PRRSV infectious clone. The infectious clone derived from pCMV-S-P129-PK17-FL that incorporate this deletion is pCMV-S-P129-PK17-nsp2.

Infectious clones that combine the NLS and nsp2 deletions were also generated within the pCMV-S-P129-PK17-FL backbone, and these were designated pCMV-S-P129-PK17-nsp2-d43/44 and pCMV-S-P129-PK17-nsp2-d43/44/46.

Example 4

Generation and Growth of Viruses on PK-9 Cells

The six infectious clones described in Example 3 were transfected into PK-9 cells to generate the six viruses as shown in Table 1. Virus was generated from these infectious clones by direct transfection of the circular plasmid into PK-9 cells using Lipofectamine 2000. Following transfection, recovered viruses were again serially passaged on PK-9

28

cells in order to further increase titers and attenuate virulence. Stocks were made for in vitro testing and in vivo evaluation as vaccine candidates. In the case of P129-PK17-FL virus derived from the non-modified pCMV-S-P129-PK17-FL infectious clone, the virus was cultured until reaching a total of 52 passages from the pig. The complete genome of this virus was sequenced at passages 24 (SEQ ID NO: 3) and 52 (SEQ ID NO:6).

Example 5

Viruses Derived from the P129-PK Passage 17 Infectious cDNA Clone have Reduced Ability to Inhibit IFN-Alpha Induction

Viruses and cells. MARC-145 and ST cells were grown in modified Eagle's medium (MEM) supplemented with 5% fetal bovine serum (FBS) and antibiotics (50 µg/ml gentamicin, 100 UI penicillin and 100 µg/ml streptomycin). The porcine alveolar macrophage ZMAC-1 cells were grown in RPMI-1640 supplemented with 10% FBS. TGE virus strain Purdue was prepared by infection of confluent ST cell monolayers at a multiplicity of 0.01 in modified Eagle's medium. The virus inoculum was removed after 1 h and cells were incubated in MEM supplemented with 2.5% FBS at 37° C. in a 5% CO₂ atmosphere. Virus was released by freezing and thawing the cell monolayers after 80% cytopathic effect was observed. The TGE viral stock was centrifuged at 3,500 rpm for 15 min at 4° C. and stored at -80° C. until use. Virus stocks (from PK-9 cells) were as follows: P129-PK-FL and P129-PK-dnsp2-d43/44/46 were at passage 8/25 (8 from the infectious clone, 25 from the pig). The other four viruses (P129-PK-d43/44, P129-PK-d43/44/46, P129-PK-dnsp2, and P129-PK-dnsp2-d43/44) were at passage 21/38. Working stocks of various PRRS viruses were prepared by making a single passage on ZMAC-1 cells, except that commercial vaccines Ingelvac PRRS MLV and Ingelvac PRRS ATP were reconstituted according to the manufacturer's instructions and used directly for infection.

Isolation of porcine PBMC. Fresh heparinized venous blood was diluted with Hank's and PBMC were isolated by density centrifugation through Ficoll-Hypaque 1077 (Sigma) gradient. After being washed twice in Hank's, the cells were suspended in RPMI medium with L-glutamine (Mediatech) supplemented with 5% fetal bovine serum (Gibco), 100 U/ml penicillin, 0.1 mg/ml streptomycin, 1 mM sodium pyruvate, 1× nonessential amino acids (Mediatech) 100 U/ml gentamicin and 250 mM 2-mercaptoethanol (Sigma).

Purification of porcine plasmacytoid dendritic cells. The purification of porcine plasmacytoid dendritic cells was done as previously described (Calzada-Nova, submitted), and was based on the characteristic expression of CD4 and CD172 by these cells (Summerfield et al., 2003). Briefly, fresh porcine PBMC were suspended in PBS with 0.5% BSA and labeled with optimal amounts of mAb recognizing porcine CD172 (74-22-15, VMRD). Following one wash, the cells were then incubated with secondary goat anti-mouse antibody conjugated to PE (Southern Biotech) and after washing, with FITC labeled anti-CD4 (74-12-4, VMRD). PDCs were sorted on a Reflection Cell Sorter (iCyt), sort gates were set on the CD4⁺/CD172^{low} population. After the sort the purity of the cells was confirmed by reanalysis. In all cases, the purity was >95%.

Assay for measurement cytokine secretion. PBMC or PDC were stimulated for 16 h (37° C., 5% CO₂) with the different stimulants or were mock-stimulated. After incuba-

tion, medium overlaying the stimulated cells was assayed for the presence of IFN- α using a sandwich ELISA prepared with monoclonal antibodies available commercially (anti-pig IFN- α mAbs K9 and F17). Briefly, Immulon II plates (Dynatech Inc.) were coated with anti-porcine IFN- α mAb F17 (PBL Laboratories) by overnight incubation at 4° C. followed by blocking with RPMI medium supplemented with 5% fetal bovine serum. After 1 hour the medium was discarded and fifty microliters of the supernatant to be tested added to the assay wells in duplicate. After a 1 h incubation the assay wells were washed 4 times and then incubated sequentially with biotin-labeled, anti-porcine IFN- α mAb K9 (PBL Laboratories), HRP-conjugated streptavidin (Zymed Laboratories), and TMB substrate (KPL). The optical density was determined with an ELISA plate reader.

Viruses derived from the P129-PK passage 17 infectious cDNA clone lack the ability to inhibit IFN- α induction. Working virus stocks were prepared from a group of four different PRRS wild-type virus isolates (P3412, P129, IND5, NADC20) utilizing the porcine alveolar macrophage cell line ZMAC-1. Additional stocks were also prepared from derivatives of the first two wild-type viruses which had been adapted to grow in cell culture by repeated passage in PK-9, FK.D4 or MARC-145 cells. Three of the four wild-type isolates (P129, IND5, NADC20) grew readily and efficiently in the ZMAC-1 cells to titers of about 10⁷ TCID₅₀/ml, while the P3412 wild-type isolate reached a titer of only 10⁵ TCID₅₀/ml. Notably, stocks of P129 viruses prepared in the ZMAC-1 cell line reached 10-fold higher titers than those obtained in the PK-9 or MARC-145 cells to which the viruses were adapted. Examination of the ability of these viruses to stimulate IFN- α secretion by PBMC revealed that with one exception (isolate P3412 clone C), a very small amount (<50 pg) of IFN- α was secreted by these cells in response to their exposure to any of the PRRS virus stocks tested, which is negligible by comparison to the abundant secretion of IFN- α (17,540 pg) produced by the same cells as a result of their exposure to the porcine coronavirus, transmissible gastroenteritis virus (TGEV).

PRRSV is not only unable to stimulate IFN- α production by porcine PBMC but actively inhibits its production. The inhibitory effects of the PRRSV stocks were determined by measuring the amount of IFN- α secreted by PBMC in response to exposure to TGEV in the presence or absence of PRRSV. As shown in Table 2, all 4 of the wild-type PRRS virus isolates tested, as well as all of the cell culture adapted derivatives, exhibited a strong inhibitory effect (>80%) on the IFN- α response of PBMC to TGEV. The analysis of a group of virus stocks derived from an infectious cDNA clone (pCMV-S-P129-PK17-FL), including full-length P129-PK-FL virus and several genetically engineered deletion mutants was conducted. As shown in Table 3, when compared to the strong inhibitory effect (95%) of the parental wild-type isolate P129 (passage 1), the P129-PK-FL virus and all deletion mutants exhibited a significantly reduced ability to inhibit the induction of IFN- α by TGEV in PBMC. To further evaluate the IFN- α phenotype of these viruses, subsequent experiments were focused on performing direct comparisons between the P129-PK-FL and P129-PK-d43/44 viruses, the parental P129 wild-type strain, and/or two commercially available modified live PRRSV vaccines produced by Boehringer Ingelheim (Ingelvac PRRS MLV and Ingelvac PRRS ATP). An additional low-passage reference isolate, NVSL-14, was also tested. As shown in Table 4, in four independent experiments, P129-PK-FL and P129-PK-d43/44 exhibited significantly lesser IFN- α inhibitory effect than the parental P129 virus, the two Ingelvac attenuated

strains, or the reference strain. In one instance, co-infection with the P129-PK-FL or P129-PK-d43/44 viruses resulted in an apparent enhancement of the IFN- α response to TGEV.

The results shown in Table 2 are indicative of the interferon- α inhibitory effect of wild-type PRRS virus and derivatives adapted to growth in cell culture. The indicated PRRS virus stocks, were grown in ZMAC-1 cells and the titer of these newly generated stocks determined using ZMAC-1 cells. The amount of IFN- α present in culture supernatants of porcine peripheral blood mononuclear cells exposed for 18 h to the indicated PRRS virus stock in the presence or absence of TGE virus was determined by ELISA. *Response to TGEV alone.

The results shown in Table 3 demonstrate the interferon- α inhibitory effect of wild-type PRRS virus P129 and its genetically engineered derivatives adapted to grow in CD163-expressing PK-9 cells. The amount of IFN- α present in culture supernatants of porcine peripheral blood mononuclear cells (PBMC) exposed for 18 h to the indicated PRRS virus stock in the presence or absence of TGE virus was determined by ELISA. na=not applicable; *Response to TGEV alone.

Table 4 shows decreased interferon- α inhibitory effect of the P129-PK-FL and P129-PK-d43/44 viruses as compared to the wild-type P129 virus and the PRRS Ingelvac vaccines. The amount of IFN- α present in culture supernatants of porcine peripheral blood mononuclear cells (PBMC) exposed for 18 h to the indicated PRRS virus stock in the presence or absence of TGE virus was determined by ELISA. na=not applicable; *Response to TGEV alone.

The plentiful amount of IFN- α secreted by PBMC in response to their exposure to TGEV is derived primarily from a subset of cells that comprise less than 0.3% of the PBMC population. This infrequent but important cell subset is composed of plasmacytoid dendritic cells (PDCs), which received this name due to characteristic plasmacytoid morphology. To further examine the IFN- α phenotype of the P129-PK-FL and the P129-PK-d43/44 viruses, a series of experiments was performed similar to those described above, except that PDC freshly isolated from PBMC to a >95% purity were utilized. As shown in FIG. 1, this series of experiments confirmed that the P129-PK-FL and P129-PK-d43/44 viruses caused negligible inhibition of IFN- α induction by TGEV. Furthermore, in one experiment an apparent enhancing effect was observed on TGEV-mediated IFN- α induction by PDCs in response to P129-PK-FL and P129-PK-d43/44 PRRS viruses. In contrast, the Ingelvac PRRS MLV virus exhibited a strong inhibitory effect on the IFN- α response, as shown in FIG. 1.

The results described in the experimental section reveal that the P129-PK-FL and P129-PK-d43/44 PRRS viruses, as well as other derivatives of the pCMV-S-P129-PK17-FL infectious cDNA clone, have a greatly reduced ability to inhibit the induction of IFN- α by TGEV in infected PBMC or PDC cells. This is in marked contrast to the IFN suppressive effect observed with wild-type (low-passage) PRRS viruses and with two commercially available modified live virus vaccines (Ingelvac PRRS MLV and Ingelvac PRRS ATP). The observation that the P129-PK-FL and P129-PK-d43/44 viruses were minimally suppressive of this important function of PDCs is potentially significant given the major role that these cells play in mediating innate immunity against virus infections.

It should also be noted that the present invention provides clinically effective commercial vaccine viruses adapted to grow on permissive cells that recombinantly express CD163 receptor, and that such viruses and vaccines are not depen-

dent on, nor were developed at any point with, historical “simian cell” culturing technology. See specifically U.S. Pat. No. 7,754,464.

Example 6

Safety and Efficacy of Vaccine Candidates

In order to evaluate their safety and efficacy as vaccines against PRRS, three of the viruses derived from the pCMV-S-P129-PK17-FL infectious cDNA clone, P129-PK-FL (passage 7/24), P129-PK-d43/44 (passage 17/34) and P129-PK-d43/44/46 (passage 17/34) were evaluated in a young pig respiratory disease model. The origin of these viruses is shown in FIG. 8, and the experimental design (treatment groups) are listed in Table 5. Low passage virulent PRRSV isolate NADC20 was used for heterologous challenge at 7 weeks of age (four week past vaccination). Control treatment groups included mock vaccine and commercial PRRS vaccine Ingelvac MLV.

Non-treated (NT) groups were as follows: NT1 pigs were sentinels to monitor the health status of source pigs. They were housed separately and were necropsied prior to PRRSV challenge. NT2 pigs were contact controls housed separately in a pen between the two pens of vaccinated pigs, a total of two per treatment group for each T02 thru T05. NT3 pigs were contact controls housed one per pen with vaccinated pigs, a total of two per treatment group (T01 thru T05). Only NT3 pigs were assigned to the T01 group.

Rectal temperatures of vaccinated animals were measured post-vaccination and compared to the T01 (mock vaccine) treatment group. The results are shown in FIG. 1. None of the vaccines induced fevers. All groups averaged less than 104° C. throughout the post-vaccination observation period.

Rectal temperatures of pigs were measured post-challenge. The results are shown in FIG. 2. Unvaccinated T01 pigs showed sustained fevers of greater than 104° C. In contrast, three of the vaccines significantly reduced post-challenge fevers. P129-PK-FL was most effective at reducing fevers.

The body weight of animals was recorded pre- and post-vaccination. The results are shown in FIG. 3. Body weights were recorded on days -1 (prior to vaccination), 10, 24, 27 (prior to challenge), and 37 of the study. In unvaccinated pigs, challenge with virulent NADC20 virus almost completely eliminated weight gain during the 10 day observation period. The vaccines negated this effect to various degrees.

The lungs of challenged animals were examined at necropsied post-challenge. The percentages of each lung involved in lesions are shown in FIG. 4. The T01 mock vaccine group averaged 25.1% lung lesion involvement. The vaccines reduced lung lesions to various degrees. P129-PK-FL was most efficacious, reducing lung lesion involvement to 1.1%.

The severity of the lung lesions was evaluated using a lung assessment score (LAS) as shown in FIG. 5. Three of the vaccines reduced LAS. P129-PK-FL reduced the mean LAS from 1.63 in the mock vaccinated group to 0.14.

Serum antibodies to PRRSV were induced by both vaccination and challenge. The IDEXX ELISA S/P ratios were measured on days 27 and 37 of the study. The results are shown in FIG. 6. Vaccination with P129-PK-FL induced the highest levels of anti-PRRS antibodies.

Viremia in the serum of challenged pigs was titrated on PAM cells. Results (TCID₅₀/mL) are given in FIG. 7. P129-PK-FL was most effective at reducing post-challenge viremia.

Although the invention has been described with reference to the above examples, and to Attachments, the entire content of each of which are incorporated by reference in their entirety, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

Example 7

Derivation of Infectious cDNA Clone pCMV-S-P129-PK17-FL from Infectious cDNA Clone pCMV-S-P129

The PRRSV infectious cDNA clone of the present invention pCMV-S-P129-PK17-FL can readily be derived from the previously described PRRSV infectious cDNA clone pCMV-S-P129 by one of ordinary skill in the art, using the technique of site-directed mutagenesis. PRRSV infectious cDNA clone pCMV-S-P129 is described in U.S. Pat. No. 6,500,662 and deposited with ATCC under accession number 203489. The DNA sequence of the PRRSV genome in this clone also available in the Genbank (NCBI) database as accession number AF494042. Site-directed mutagenesis kits are commercially available from a number of suppliers, and are capable of making numerous simultaneous nucleotide changes at multiple sites in large plasmids. Such kits include, but are not limited to, Change-IT™ Multiple Mutation Site Directed Mutagenesis Kit (Affymetrix/USB), QuikChange Lightning Multi Site-Directed Mutagenesis Kit (Agilent Technologies—Stratagene Products), and AMAP Multi Site-directed Mutagenesis Kit (MBL International).

A list of nucleotide changes between PRRSV infectious cDNA clone pCMV-S-P129 (available from ATCC) and the PRRSV infectious cDNA clone of the present invention pCMV-S-P129-PK17-FL is presented in Table 8. All changes are in the protein coding regions of the genome. There are a total of 74 nucleotide changes, which can be introduced into the pCMV-S-P129 infectious clone using 74 mutagenic primers and multiple sequential reactions with a commercial site-directed mutagenesis kit, yielding a plasmid molecule that is identical in sequence to pCMV-S-P129-PK17-FL described herein. In actuality, one can get the same result with fewer than 74 mutagenic primers, since clusters of mutations within about 50-60 nucleotides of each other can be changed using a single mutagenic primer. For example, nucleotides 735, 750, and 756 can be changed using a single mutagenic primer, as can nucleotides 965, 992, and 1009. Thus the number of primers is reduced to about 60.

Of the 74 nucleotide changes, the majority (42) are synonymous or “silent”, meaning they encode the same amino acid. These nucleotide changes are unlikely to have any measurable effect on the interferon induction or inhibition phenotype of the virus. The remaining 32 nucleotide changes are non-synonymous or “non-silent”, and result in amino acid changes in viral proteins. These 32 nucleotide changes are predicted to be directly responsible for the interferon induction/inhibition phenotype of the virus, and should be changed in order to convert the virus encoded by the infectious clone pCMV-S-P129 to the same interferon phenotype as the shown by the virus encoded by infectious clone pCMV-S-P129-PK17-FL. Such a change would

require at most 32 mutagenic primers, less if one takes into account the clustering of some of the relevant nucleotides.

Example 8

De Novo Synthesis of Infectious cDNA Clone
pCMV-S-P129-PK17-FL

As an alternative to site-directed mutagenesis, the PRRS viral genome of the present invention can be chemically synthesized de novo, with appropriate 5' and 3' adaptor

TABLE 1

Infectious clone	Virus
pCMV-S-P129-PK17-FL	P129-PK-FL
pCMV-S-P129-PK17-d43/44	P129-PK-d43/44
pCMV-S-P129-PK17-d43/44/46	P129-PK-d43/44/46
pCMV-S-P129-PK17-nsp2	P129-PK-nsp2
pCMV-S-P129-PK17-nsp2-d43/44/46	P129-PK-nsp2-d43/44
pCMV-S-P129-PK17-nsp2-d43/44/46	P129-PK-nsp2-d43/44/46

TABLE 2

SAMPLE	PRRS v stock	CLONE	Passage/Cell type	Estimated PRRS virus titer (TCID ₅₀) in original stock	PRRS virus titer (TCID ₅₀) in PRRS virus stocks generated in ZMAC-1 cells	IFN-α (ng/ml) produced by PBMC in response to PRRSv alone	IFN-α (ng/ml) produced by PBMC in response to PRRSv + TGEv	Inhibition of IFN-α response to TGEv (%)
1	P3412	wt	0 (serum)	nd	10 ⁵	<0.04	<0.04	99
2	P3412	A	41/PK	10 ³	10 ³	<0.04	1.166	93
3	P3412	C	41/PK	10 ³	10 ³	0.464	3.376	81
4	P3412	A	43/FK	10 ⁵	10 ⁶	<0.04	<0.04	99
5	P3412	B	43/FK	10 ⁵	10 ⁵	<0.04	1.86	89
6	P129	wt	0 (serum)	nd	10 ⁷	<0.04	0.327	98
7	P129	A	63/PK	10 ⁴	10 ⁸	<0.04	<0.04	99
8	P129	B	63/PK	10 ⁴	10 ⁸	<0.04	2.3	87
9	P129	A	60/FK	10 ⁵	10 ⁶	0.05	2.151	88
10	P129	B	60/FK	10 ⁵	10 ⁶	<0.04	<0.04	99
11	P129	A-1	51/MARC	10 ⁵	10 ⁸	<0.04	0.686	96
12	P129	A-1	151/MARC	10 ⁵	10 ⁸	<0.04	0.04	99
13	NADC 20	wt	0 (serum)	nd	10 ⁷	<0.04	<0.04	99
14	IND5	wt	0 (serum)	nd	10 ⁷	<0.04	<0.04	99
—	Mock	—	—	na	na	0	17.54*	na

sequences, and cloned into the plasmid backbone used for PRRS infectious cDNA clone pCMV-S-P129 (available from ATCC as accession number 203489) or a similar plasmid backbone. Custom synthesis of genes greater than 50 kb in length (the PRRSV genome is about 15.5 kb) is available as a commercial service from numerous vendors, including (but not limited to): GenScript, DNA 2.0, and Bio Basic Inc. The synthetic viral genome is directionally cloned into the pCMV-S vector by replacing the viral genome in the infectious clone pCMV-S-P129 using the 5' PacI and 3' SpeI restriction enzyme sites that flank the genome. In order to cut the synthetic genome, a 24-nucleotide extension

(5' -GCAGAGCTCGTTAATTAACCGTC-genome-3' ,

which includes the underlined PacI site) is built into the 5' end of the synthetic genome, and an 83-nucleotide extension (5'-genome-AAAAAAAAAAAAAAAAAAAAAAAAATGCAT-ATTTAAATCCCAAGCCGAATTCAGCACA CTGGCGGCCGTTACTAGTGAGCGGCCGC-3', which includes the underlined SpeI site) is built into the 3' end of the synthetic genome. After cutting the plasmid and synthetic genome with PacI and SpeI, the appropriate fragments are purified, joined using DNA ligase, and transformed into *Escherichia coli* for screening and propagation using standard cloning techniques well known to persons of ordinary skill in the art.

TABLE 3

Experi-ment	PRRS virus stock	IFN-α (ng/ml) produced by PBMC in response to PRRSv alone	IFN-□α (ng/ml) produced by PBMC in response to PRRSv + TGEv	Inhibition of TGEv induced IFN-α response (%)
1	P129	0.09	0.64	95
	P129-PK-FL	0.53	7.37	38
	P129-PK-d43/44	0.85	8.59	28
	P129-PK-d43/44/46	1.01	7.65	36
	P129-PK-dnsp2d43/44	1.34	10.28	24
	P129-PK-dnsp2d43/44/46	0.38	8.28	31
	P129-PK-dnsp2	0.04	4.86	60
	Mock	na	12.16*	na

TABLE 4

Experi-ment	PRRS virus stock	IFN-α (ng/ml) produced by PBMC in response to PRRSv alone	IFN-□α (ng/ml) produced by PBMC in response to PRRSv + TGEv	Inhibition of TGEv induced IFN-α response (%)
1	P129	0.04	9.75	81
	P129-PK-FL	0.07	60.22	0

35

TABLE 4-continued

Experi- ment	PRRS virus stock	IFN- α (ng/ml) produced by PBMC in re- sponse to PRRSv alone	IFN- α (ng/ml) produced by PBMC in re- sponse to PRRSv + TGEv	Inhibition of TGEv induced IFN- α response (%)
2	P129-PK- d43/44	0.13	70.10	0
	Ingelvac PRRS	0.04	13.05	74
	Ingelvac PRRS ATP	0.04	9.10	82
	NVSL-14	0.01	13.35	74
	Mock	na	50.06*	na
	P129-PK-FL	0.126	13.52	26
	P129-PK- d43/44	0.127	17.18	6
	Ingelvac PRRS	0.04	3.09	83
	Ingelvac PRRS ATP	0.04	3.11	83
	Mock	na	18.21*	na
3	P129-PK-FL	0.04	8.28	6
	P129-PK- d43/44	0.06	8.26	6
	Ingelvac PRRS	0.04	3.56	60
	Ingelvac PRRS ATP	0.04	4.15	53
	Mock	na	8.84*	na
4	P129-PK-FL	0.05	12.97	7
	P129-PK- d43/44	0.05	13.57	3
	Ingelvac PRRS	0.04	6.07	57
	Ingelvac PRRS ATP	0.04	5.30	62
	Mock	na	13.95*	na

TABLE 5

TX	IVP	Passage or Serial #	Cell Line	Regimen	Volume per IM Dose	# of Pigs
NT1*	NA	NA	NA	NA	NA	3
NT2*	NA	NA	NA	NA	NA	8
NT3*	NA	NA	NA	NA	NA	10
T01	Mock	NA	NA	Day 0	2 mL	12
T02	P129-PK- d43/44	17/34	PK9	Day 0	2 mL	12
T03	P129-PK- d43/44/46	17/34	PK9	Day 0	2 mL	12
T04	P129-PK-FL	7/24	PK9	Day 0	2 mL	12
T05	BI (Ingelvac MLV)	NA	Monkey kidney	Day 0	2 mL	12

TABLE 6

Genome position	Passage 0 nucleotide	Passage 17 nucleotide	Affected viral protein	Amino acid position	Passage 0 amino acid	Passage 17 amino acid
407	C	T	Nsp1a	72	P	P
612	C	T	Nsp1a	141	H	Y
735	G	A	Nsp1b	2	D	N
750	A	G	Nsp1b	7	S	G
756	G	A	Nsp1b	9	D	N
992	A	T	Nsp1b	87	E	D
1009	G	A	Nsp1b	93	C	Y
1096	C	A	Nsp1b	122	P	H
1215	C	T	Nsp1b	162	L	L
1620	G	A	Nsp2	94	A	T
1786	C	A	Nsp2	149	T	K
1793	C	T	Nsp2	151	G	G

36

TABLE 6-continued

Genome position	Passage 0 nucleotide	Passage 17 nucleotide	Affected viral protein	Amino acid position	Passage 0 amino acid	Passage 17 amino acid
1808	T	C	Nsp2	156	D	D
1841	C	T	Nsp2	167	C	C
2106	A	G	Nsp2	256	I	V
2164	T	G	Nsp2	275	M	R
2185	T	C	Nsp2	282	M	T
2318	A	G	Nsp2	326	S	S
2403	G	T	Nsp2	355	V	L
2591	G	A	Nsp2	417	L	L
2804	C	T	Nsp2	488	D	D
3019	A	G	Nsp2	560	Y	C
3074	T	C	Nsp2	578	S	S
3167	C	T	Nsp2	609	D	D
3214	G	A	Nsp2	625	R	K
3563	C	T	Nsp2	741	I	I
3740	A	G	Nsp2	800	A	A
4154	T	C	Nsp2	938	C	C
4477	A	C	Nsp3	72	K	T
4643	A	G	Nsp3	127	V	V
4705	T	C	Nsp3	148	V	A
4736	C	T	Nsp3	158	P	P
5231	C	T	Nsp3	323	I	I
5324	C	T	Nsp3	354	L	L
5393	G	A	Nsp3	377	L	L
5498	A	G	Nsp3	412	L	L
5851	C	A	Nsp4	84	A	E
5855	T	C	Nsp4	85	D	D
5909	C	T	Nsp4	103	V	V
5917	G	A	Nsp4	106	S	N
5985	C	A	Nsp4	129	L	I
6505	C	T	Nsp5	98	A	V
6644	T	C	Nsp5	144	F	F
6653	T	C	Nsp5	147	R	R
7419	G	A	Nsp7	217	D	N
8032	A	G	Nsp9	162	A	A
8074	C	T	Nsp9	176	G	G
8200	A	G	Nsp9	218	G	G
8593	T	C	Nsp9	349	P	P
8831	G	A	Nsp9	429	V	I
8911	T	C	Nsp9	455	N	N
9160	A	G	Nsp9	538	E	E
9568	A	G	Nsp9	674	L	L
9714	T	C	Nsp10	38	I	T
10,271	C	T	Nsp10	224	L	L
10,627	T	C	Nsp10	342	V	V
11,265	G	A	Nsp11	114	G	E
11,512	T	C	Nsp11	196	S	S
11,913	T	C	Nsp12	107	I	T
12,487	A	T	ORF2a	144	E	V
12,876	C	T	ORF3	66	A	V
12,949	A	G	ORF3	90	L	L
13,575	G	A	ORF4	117	V	V
13,857	T	G	ORF5	29	V	G
13,869	A	G	ORF5	33	N	S
13,872	C	G	ORF5	34	T	S
14,102	G	A	ORF5	111	V	I
14,143	C	T	ORF5	124	V	V
14,257	G	A	ORF5	162	E	E
14,287	C	T	ORF5	172	N	N
14,379	T	C	ORF6	7	D	D
14,546	T	C	ORF6	63	V	A
14,578	A	G	ORF6	74	T	A
14,780	C	T	ORF6	141	T	I
14,932	T	C	ORF7	20	N	N
14,973	G	A	ORF7	34	S	N
15,124	T	C	ORF7	84	N	N
15,288	G	A	3'UTR	—	—	—

TABLE 7

ORF or nsp	Nucleotide differences	Amino acid differences	Non-conservative amino acid differences
Nsp1a	2	1	1
Nsp1b	7	6	5
Nsp2	19	8	5

TABLE 7-continued

ORF or nsp	Nucleotide differences	Amino acid differences	Non-conservative amino acid differences
Nsp3	8	2	1
Nsp4	5	3	2
Nsp5	3	1	0
Nsp6	0	0	0
Nsp7	1	1	1
Nsp8	0	0	0
Nsp9	8	1	0
Nsp10	3	1	1
Nsp11	2	1	1
Nsp12	1	1	1
ORF2a	1	1	1
ORF2b	0	0	0
ORF3	2	1	0
ORF4	1	0	0
ORF5	7	4	2
ORF6	4	3	2
ORF7	3	1	1

TABLE 8

Genome position	Passage 10 on MARC-145 cells nucleotide	Passage 17 on PK-9 cells nucleotide	Affected viral protein	Amino acid position	Passage 10 (on MARC-145 cells) amino acid	Passage 17 (on PK-9 cells) amino acid
407	C	T	Nsp1a	72	P	P
612	C	T	Nsp1a	141	H	Y
735	G	A	Nsp1b	2	D	N
750	A	G	Nsp1b	7	S	G
756	G	A	Nsp1b	9	D	N
965	T	C	Nsp1b	78	G	G
992	A	T	Nsp1b	87	E	D
1009	G	A	Nsp1b	93	C	Y
1096	C	A	Nsp1b	122	P	H
1215	C	T	Nsp1b	162	L	L
1376	A	G	Nsp2	12	A	A
1395	T	C	Nsp2	19	C	R
1871	A	G	Nsp2	177	L	L
2185	T	C	Nsp2	282	M	T
2235	G	A	Nsp2	299	D	N
2403	G	T	Nsp2	355	V	L
2731	A	G	Nsp2	464	Y	C
2804	C	T	Nsp2	488	D	D
2918	T	C	Nsp2	526	S	S
3019	A	G	Nsp2	560	Y	C
3067	G	A	Nsp2	576	G	E
3074	T	C	Nsp2	578	S	S
3214	G	A	Nsp2	625	R	K
3256	T	C	Nsp2	639	L	S
3563	C	T	Nsp2	741	I	I

TABLE 8-continued

Genome position	Passage 10 on MARC-145 cells nucleotide	Passage 17 on PK-9 cells nucleotide	Affected viral protein	Amino acid position	Passage 10 (on MARC-145 cells) amino acid	Passage 17 (on PK-9 cells) amino acid
3740	A	G	Nsp2	800	A	A
4154	T	C	Nsp2	938	C	C
4477	A	C	Nsp3	72	K	T
4643	A	G	Nsp3	127	V	V
4705	T	C	Nsp3	148	V	A
4736	C	T	Nsp3	158	P	P
4784	C	T	Nsp3	174	V	V
5231	C	T	Nsp3	323	I	I
5324	C	T	Nsp3	354	L	L
5498	A	G	Nsp3	412	L	L
5855	T	C	Nsp4	85	D	D
5862	G	A	Nsp4	88	A	T
5985	C	A	Nsp4	129	L	I
6155	T	C	Nsp4	185	D	D
6505	C	T	Nsp5	98	A	V
6776	A	G	Nsp7	2	L	L
7419	G	A	Nsp7	217	D	N
7521	T	C	Nsp7	251	S	P
8032	A	G	Nsp9	162	A	A
8074	C	T	Nsp9	176	G	G
8200	A	G	Nsp9	218	G	G
8263	T	C	Nsp9	239	S	S
8485	T	C	Nsp9	313	H	H
8593	T	C	Nsp9	349	P	P
8831	G	A	Nsp9	429	V	I
8911	T	C	Nsp9	455	N	N
9022	A	G	Nsp9	492	L	L
9714	T	C	Nsp10	38	I	T
9934	C	T	Nsp10	111	N	N
10,237	G	A	Nsp10	212	L	L
10,271	C	T	Nsp10	224	L	L
10,333	T	C	Nsp10	244	L	L
10,520	A	G	Nsp10	307	M	V
10,627	T	C	Nsp10	342	V	V
10,847	A	C	Nsp10	416	I	L
10,867	C	T	Nsp10	422	F	F
10,936	C	T	Nsp11	4	S	S
11,512	T	C	Nsp11	196	S	S
12,949	A	G	ORF3	90	L	L
13,452	C	T	ORF4	76	P	P
13,575	G	A	ORF4	117	V	V
13,843	T	G	ORF5	24	C	W
13,860	G	A	ORF5	30	S	N
14,287	C	T	ORF5	172	N	N
14,481	A	G	ORF6	41	L	L
14,546	T	C	ORF6	63	V	A
14,578	A	G	ORF6	74	T	A
14,780	C	T	ORF6	141	T	I
14,932	T	C	ORF7	20	N	N

TABLE 9

Amino acid changes responsible for reduced interferon inhibition and attenuation of virulence.					
Genome Position (nt)	ORF and position (aa)	Nsp and position (aa)	Passage 0 amino acid	Passage 17 amino acid	Passage 52 amino acid
735	ORF1a: 182	Nsp1b: 2	AMAD <u>V</u> YD	AMAN <u>V</u> YD	AMAN <u>V</u> YD
756	ORF1a: 189	Nsp1b: 9	ISHD <u>A</u> VM	IGHN <u>A</u> VM	IGHN <u>A</u> VM
992	ORF1a: 267	Nsp1b: 87	TVPE <u>G</u> NC	TVPD <u>G</u> NC	TVPD <u>G</u> NC
1009	ORF1a: 273	Nsp1b: 93	CWW <u>C</u> LFD	CWW <u>Y</u> LFD	CWW <u>Y</u> LFD
1096	ORF1a: 302	Nsp1b: 122	HGV <u>P</u> GKY	HGVH <u>G</u> KY	HGVH <u>G</u> KY

TABLE 9-continued

Amino acid changes responsible for reduced interferon inhibition and attenuation of virulence.					
Genome Position (nt)	ORF and position (aa)	Nsp and position (aa)	Passage 0 amino acid	Passage 17 amino acid	Passage 52 amino acid
2106	ORF1a: 639	Nsp2: 256	AAKIDQY	AAKVDQY	AAKVDQY
2185	ORF1a: 665	Nsp2: 282	PSAMDTS	PSATDTS	PSATDTS
2403	ORF1a: 738	Nsp2: 355	LVS ~~V~~ LSK	LNS ~~L~~ LSK	LNS ~~L~~ LSK
3019	ORF1a: 943	Nsp2: 560	APMYQDE	APMCQDE	APMCQDE
4477	ORF1a: 1429	Nsp3: 72	CAPK ~~G~~ GMD	CAPT ~~G~~ GMD	CAPT ~~G~~ GMD
4705	ORF1a: 1505	Nsp3: 148	PKV ~~V~~ KVS	PKV ~~A~~ KVS	PKV ~~A~~ KVS
5985	ORF1a: 1932	Nsp4: 129	AGE ~~L~~ VG ~~V~~	AGE ~~I~~ VG ~~V~~	AGE ~~I~~ VG ~~V~~
7419	ORF1a: 2410	Nsp7: 217	ADFD ~~P~~ PEK	ADFN ~~P~~ PEK	ADFN ~~P~~ PEK
8831	ORF1a/1b: 2881	Nsp9: 429	QTPV ~~L~~ LGR	QTP ~~I~~ LGR	QTP ~~I~~ LGR
13,857	ORF5: 29	—	AVL ~~V~~ NAN	AVL ~~G~~ NAN	AVL ~~G~~ NAN
14,578	ORF6: 74	—	VAL ~~T~~ MGA	VAL ~~A~~ MGA	VAL ~~A~~ MGA
14,780	ORF6: 141	—	PGS ~~T~~ TVN	PGS ~~I~~ TVN	PGS ~~I~~ TVN

TABLE 10

Amino acid changes responsible for reduced interferon inhibition and attenuation of virulence					
Genome Position (nt)	ORF and position (aa)	Nsp and position (aa)	Passage 0 amino acid	Passage 17 amino acid	Passage 52 amino acid
735	ORF1a: 182	Nsp1b: 2	AMAD ~~V~~ YD	AMAN ~~V~~ YD	AMAN ~~V~~ YD
756	ORF1a: 189	Nsp1b: 9	ISHD ~~A~~ VM	IGHN ~~A~~ VM	IGHN ~~A~~ VM
1009	ORF1a: 273	Nsp1b: 93	CWW ~~C~~ LFD	CWW ~~Y~~ LFD	CWW ~~Y~~ LFD
1096	ORF1a: 302	Nsp1b: 122	HGVP ~~G~~ KY	HGVH ~~G~~ KY	HGVH ~~G~~ KY
2185	ORF1a: 665	Nsp2: 282	PSAMDTS	PSATDTS	PSATDTS
3019	ORF1a: 943	Nsp2: 560	APMYQDE	APMCQDE	APMCQDE
4477	ORF1a: 1429	Nsp3: 72	CAPK ~~G~~ GMD	CAPT ~~G~~ GMD	CAPT ~~G~~ GMD
4705	ORF1a: 1505	Nsp3: 148	PKV ~~V~~ KVS	PKV ~~A~~ KVS	PKV ~~A~~ KVS
7419	ORF1a: 2410	Nsp7: 217	ADFD ~~P~~ PEK	ADFN ~~P~~ PEK	ADFN ~~P~~ PEK

Example 9

Vaccine Consisting of P129-PKC12-FL Virus is Safe for Use in 1 Day Old Pigs and Efficacious for at Least 26 Weeks

Existing PRRS modified live vaccines are only recommended for use in pigs two weeks of age or older. Studies were conducted to determine if the attenuated P129-PKC12-FL virus at passage 57 (2.13 log₁₀ TCID₅₀ in a 2 mL dose) is safe for use in 1-day old neonatal pigs, and sufficiently immunogenic to provide protection against a virulent heterogeneous challenge at up to 6 months (26 weeks) follow-

55 ing vaccination. (this is essentially the same as the passage 52 virus, as described in SEQ ID No: 6).

A total of 22 of 24 PRRSV seronegative pregnant sows sourced produced healthy piglets. These pigs (piglets) were 60 administered a single 2.0 mL dose of the Mock Vaccine or the P129-PKC12-FL virus vaccine as an intramuscular injection at approximately 1 day of age (Day 0) according to an allotment. All healthy piglets in a litter/farrowing crate received the same Mock Vaccine (11 litters, 100 piglets) or 65 P129-PKC12-FL virus vaccine (11 litters, 91 piglets) on the same day.

Mock vaccinated sows and piglets remained PRRSV negative throughout the vaccination period. No confounding disease factors were detected. The primary variables used to demonstrate safety in piglets vaccinated at 1 day of age were clinical observations post-vaccination. Serology was used to confirm successful vaccination of all piglets.

Clinical observations were observed and recorded for all piglets on Days 1 thru 10 post vaccination. Of the 100 pigs (piglets) administered the control product (T01) and 91 piglets administered the test product (T02), 15 and 14 pigs, respectively were observed to be not normal (Table 11, referring to the tables as numbered within this specific example). Piglets observed as not normal were further noted as having abnormal general condition and/or depression.

TABLE 11

Number of Pigs Ever Observed with Clinical Signs Following a Vaccination at One Day of Age with a Modified Live PRRSV Vaccine or Control [Number of animals observed with an abnormal health condition (% animals observed with an abnormal condition)]							
Treatment Group	Not Normal	General Condition	Depression	Respiratory Distress	Cough	Sneeze	Other
Mock Vaccine	15 (15.3)	15 (15.3)	5 (5.2)	0	0	0	0
P129- PKC12- FL Vaccine	14 (15.4)	14 (15.4)	5 (5.6)	0	0	0	0

IDEXX ELISA results confirmed all pigs were negative for PRRSV prior to vaccination (S/P ratio <0.4) and all mock vaccine controls remained serologically negative during the vaccination phase of this study. Following vaccination, all pigs in the P129-PKC12-FL Vaccine group were sero-positive (S/P ratio >0.4) by Day 21 or 22 (Table 12 below).

TABLE 12

Serum Geometric Mean PRRSV Titers (IDEXX Elisa) Following a Vaccination at One Day of Age with a Modified Live PRRSV Vaccine or Control [Mean titer (Animals positive*/total animals)]			
Treatment Group	Day 0	Day 10/11	Day 21/22
Mock Vaccine	0.002 (0/100)	0.000 (0/86)	0.001 (0/85)
P129-PKC12-FL Vaccine	0.002 (0/91)	1.322 (76/85)	2.412 (81/81)

The data supports the conclusion that attenuated P129-PKC12-FL virus at passage 57 is safe when administered as

a single 2 mL IM dose to piglets produced by seronegative sows at one day of age when observed though weaning at 21 or 22 days of age.

Prior to challenge, mock vaccinated and P129-PKC12-FL virus vaccinated piglets were re-housed in pens (2 piglets from the same vaccination group per pen) in rooms, such that each room contained 12 pens from each vaccination group. Challenged was with the virulent heterologous PRRS isolate NADC20 at either 7, 18, or 26 weeks post-vaccination. A challenge dose equaled 4.0 mL (1.0 mL per nostril plus a 2.0 mL intramuscular injection) of NADC20 stock

solution at 2.27 log₁₀ TCID₅₀/mL (2.87 log₁₀ TCID₅₀/4 mL dose). NADC-20 is a very virulent genotype 2 PRRS virus, provided by Dr. Kelly Lager of the National Animal Disease Center, USDA (Ames, Iowa). The NADC-20 ORF5 amino acid sequence is 94.5% identical to P129-PKC12-FL.

The primary variable in determining prevention of disease was percent lung with lesions. Lung lesions were scored at necropsy (10 days post-challenge) such that the percentage of consolidation for each lobe (left cranial, left middle, left caudal, right cranial, right middle, right caudal, and accessory) were scored and recorded as percent of lobe observed with lesions.

At 7 weeks post-vaccination, percent lung with lesions was significantly greater in the mock vaccinated pigs compared to P129-PKC12-FL virus vaccinated piglets (P≤0.0001) (Table 13).

TABLE 13

Back Transformed Least Square Means Percent Lung with Lesions Following a PRRSV NADC20 Challenge of 7-Week-Old Piglets Previously Vaccinated at One-Day of Age with a Modified Live PRRSV Vaccine or Mock Vaccine						
Treatment Group	Number of Pigs	% Lung with Lesions	Standard Error	Lower 95% Confidence Interval	Upper 95% Confidence Interval	Range
Mock Vaccine	24	43.9	3.48	36.3	51.7	18.5-68.5
P129- PKC12-FL Vaccine	22	0.7	0.29	0.2	1.5	0-8.25

At 18 weeks post-vaccination, percent lung with lesions was significantly greater in the mock vaccinated piglets compared to P129-PKC12-FL virus vaccinated piglets ($P\leq 0.0001$) (Table 14).

TABLE 14

Back Transformed Least Square Means Percent Lung with Lesions Following a PRRSV NADC20 Challenge of 18-Week-Old Piglets Previously Vaccinated at One-Day of Age with a Modified Live PRRSV Vaccine or Control						
Treatment Group	Number of Pigs	% Lung with Lesions	Standard Error	Lower 95% Confidence Interval	Upper 95% Confidence Interval	Range
Mock Vaccine	23	21.1	3.70	13.6	29.7	1.9-63.0
P129-PKC12-FL Vaccine	20	1.0	0.43	0.3	2.1	0-5.95

At 26 weeks post-vaccination, percent lung with lesions was significantly greater in the mock vaccinated pigs compared to P129-PKC12-FL virus vaccinated piglets ($P\leq 0.0001$) (Table 15).

TABLE 15

Back Transformed Least Square Means Percent Lung with Lesions Following a PRRSV NADC20 Challenge of 26-Week-Old Piglets Previously Vaccinated at One-Day of Age with a Modified Live PRRSV Vaccine or Control						
Treatment Group	Number of Pigs	% Lung with Lesions	Standard Error	Lower 95% Confidence Interval	Upper 95% Confidence Interval	Range
Mock Vaccine	24	17.7	2.59	12.4	23.8	4.5-51
P129-PKC12-FL Vaccine	24	1.2	0.72	0.1	3.3	0-20

The results indicate that attenuated vaccine virus P129-PKC12-FL is safe in 1-day old piglets, and is capable of inducing a potent immunological response with an exceptional duration of immunity. A single dose protects 1-day old piglets from a virulent heterologous PRRS challenge for at least 26 weeks.

These properties of safety in 1-day old piglets and 26 week duration of PRRS immunity is useful for multivalent combination swine vaccines, such as bivalent PRRSV/*Mycoplasma hyopneumoniae* (*M. hyo*) vaccines, bivalent PRRSV/Porcine Circovirus type 2 (PCV2) vaccines, and trivalent PRRSV/*M. hyo*/PCV2 vaccines, as well as for monovalent PRRSV vaccines.

Example 10

Vaccine Consisting of P129-PKC12-FL Virus Provides an Early Onset of Protective Immunity

Existing modified live PRRS vaccines are recommended for vaccination at least 3 to 4 weeks prior to exposure to virulent PRRS strains. This time interval is believed to be necessary in order to establish protective immunity. The study described here demonstrates significant protection

against a virulent heterologous PRRS virus challenge delivered only 14 days following vaccination with the P129-PKC12-FL virus vaccine and two other commercial PRRS vaccines.

During the vaccination phase, treatment groups of 18 pigs (at 3 weeks of age) were housed in four separate rooms in pens of 6 animals each. Pigs (piglets) were administered a single intramuscular injection of the Mock Vaccine (2 mL),

the attenuated P129-PKC12-FL passage 57 virus vaccine ($3.62 \log_{10}$ TCID₅₀ in a 2 mL dose), Ingelvac PRRS MLV vaccine, or Ingelvac PRRS ATP vaccine, at approximately 3 weeks of age (Day 0) according to manufacturer's instructions.

Prior to challenge, all remaining animals were re-housed in pens of 3 animals each, with one empty pen between each occupied pen, such that multiple pens of animals from each treatment group were housed in each of four rooms. Challenged was with the virulent heterologous PRRS isolate NADC20 at approximately 5 weeks of age (Day 14). A challenge dose equaled 4.0 mL (1.0 mL per nostril plus a 2.0 mL intramuscular injection) of NADC20 stock solution at $2.07 \log_{10}$ TCID₅₀/mL ($2.67 \log_{10}$ TCID₅₀/4 mL dose).

The primary variable in determining reduction of disease was percent lung lesions in the vaccinated groups in relation to mock vaccine group. Significant differences were found between all vaccinated groups (P129-PKC12-FL, $P=0.0177$; Ingelvac PRRS ATP, $P=0.0255$; Ingelvac PRRS MLV, $P=0.0137$) when compared to the mock vaccinated group. No significant differences were found when comparing vaccinated groups to each other (Table 16).

TABLE 16

Percent Lung with Lesions Following a PRRSV NADC20 Challenge of Five-Week-Old Pigs Previously Vaccinated with a Modified Live PRRSV Vaccine or Mock Vaccine.				
Treatment Group	Number of Pigs	% Lung with Lesion	Standard Error	Range
Mock Vaccine	18	46.1	10.12	1.4-88.4
P129-PKC12-FL Vaccine	18	17.5	7.71	0.23-76.6
Ingelvac PRRS ATP Vaccine	18	18.9	7.96	0.18-88.96
Ingelvac PRRS MLV Vaccine	17	16.0	7.62	0.45-71.2

These results demonstrate that it may be a general property of modified live PRRS virus vaccines to induce partial immunity and a reduction of disease by 14 days post-vaccination. This property may result from a combination of early acquired immunity (e.g. specific antibodies and cytotoxic T cells), innate immunity (e.g. induced interferons and natural killer cells), and/or competition between the vaccine virus and the challenge virus for limited numbers of permissive host cells (e.g. alveolar macrophages) in the pig (piglet). Regardless of the mechanism(s), this property can be utilized to protect pigs from disease associated with natural or intentional PRRS infection (such as intentional exposure of incoming replacement gilts with an endemic farm strain of virulent PRRS).

Thus, as aforementioned, this property of early onset of PRRS immunity is useful for multivalent combination swine vaccines, such as bivalent PRRSV/*Mycoplasma hyopneumoniae* (*M. hyo*) vaccines, bivalent PRRSV/Porcine Circovirus type 2 (PCV2) vaccines, and trivalent PRRSV/*M. hyo*/PCV2 vaccines, as well as for monovalent PRRSV

vaccines. As to the components of such vaccines, useful in the practice of the present invention (i.e. to provide early and safe vaccination as early as when the piglet is 1 day of age, optionally with onset of immunity at two weeks thereafter), reference is made to all the combination vaccine components as described in U.S. provisional application 61/620,189 of Niztel et al, entitled “PCV/*Mycoplasma hyopneumoniae*/PRRSZ Combination Vaccine”, filed Apr. 4, 2012, the complete and entire disclosure of which is incorporated by reference herein, as if set forth in its entirety.

In regard of specific PRRS vaccines (or PRRS vaccine strains) that may be used in the practice of the present invention (i.e. to provide early and safe vaccination as early as when the piglet is 1 day of age, optionally with onset of immunity at two weeks thereafter), attention is directed to Table 1 of Murtaugh et al., Vaccine, vol 29, pp. 8192-8204, (2011), see Page 8196 thereof, where numerous such viruses/vaccines are identified, including, without limitation, Ingelvac PRRS MLV, Ingelvac PRRS ATP, and Suvaxyn PRRS (derived from Iowa State strain ISU-55). It should be noted that vaccines providing the above-mentioned performance characteristics are also expected to provide a duration of immunity period of about 6 months.

Deposit of Biological Materials

The following biological materials (see also U.S. Pat. No. 6,500,662) were deposited with the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, Va., 20110-2209, USA on Nov. 19, 1998, and were assigned the following accession numbers.

Plasmid pT7P129A, accession number 203488

Plasmid pCMV-S-P129, accession number 203489

The complete text and disclosure of the following United States patents is incorporated herein by reference, as if fully set forth: U.S. Pat. Nos. 6,500,662, and 7,618,797.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 59

<210> SEQ ID NO 1

<211> LENGTH: 15450

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: P129-PK-FL passage 17 complete genome (15,450 nt) full length infectious clone

<400> SEQUENCE: 1

atgacgtata ggtggttgct ctatgccacg gcatttgtat tgtcaggagc tgtgaccatt 60

ggcacagccc aaaacttgct gcacggaaaa cgcccttctg tgacagcctt cttcagggga 120

gcttaggggt ctgtccctag caccttgctt ctggagttgc actgctttac ggtctctcca 180

cccctttaac catgtctggg atacttgatc ggtgcacgtg cacccecaat gccagggtgt 240

ttatggcgga gggccaagtc tactgcacac gatgtctcag tgcacggtct ctcttctctc 300

tgaatctcca agttctgag cttgggggtgc tgggcctatt ttataggccc gaagagccac 360

tccggtggac gttgccacgt gcattcccca ctgtcgagtg ctcccctgcc ggggcctgct 420

ggctttctgc gatctttcca attgcacgaa tgaccagtgg aaacctgaac tttcaacaaa 480

gaatggtgcg ggttgacgct gagatttaca gagccggcca actcaccctt gcagttttga 540

aggctctaca agtttatgaa cgggggtgtc gctggtagcc cattgtcgga cctgtccctg 600

gagtggccgt ttacgccaac tccctacatg tgagtgacaa acctttcccg ggagcaactc 660

-continued

atgtgttaac caacctaccg ctcccgacaga ggcccaagcc tgaagacttt tgcccttttg	720
agtgtgctat ggctaacgtc tatgacattg gccataacgc cgtcatgtat gtggccagag	780
ggaaagtctc ctggggccct cgtggcgggg atgaagtga atttgaaacc gtccccgaag	840
agttgaagtt gattgcgaac cgactccaca tctccttccc gccccaccac gcagtggaca	900
tgtctgagtt tgccctcata gcccctggga gtggtgtctc cttgcgggtc gagcaccaac	960
acggctgcct tcccgcgtgat actgtccctg atgggaactg ctggtggtac ttgtttgact	1020
tgctcccacc ggaagttcag aataaagaaa ttcgccgtgc taaccaattt ggctatcaaa	1080
ccaagcatgg tgtccatggc aagtacctac agcggaggct gcaagttaat ggtctccgag	1140
cagtgactga tacagatgga cctattgtcg tacagtactt ctctgttagg gagagttgga	1200
tccgccactt cagattggcg gaagaacctc gcctccctgg gtttgaagac ctctcagaa	1260
taagggtaga gcctaatacg tcgccattgg gtggcaaggg tgaaaaaatc ttccggtttg	1320
gcagtcacaa gtggtacggc gctggaaaga gagcaaggag agcacgctct ggtgcgactg	1380
ccacggctgc tcaccgcgct ttgcccgctc gcgaagccca gcaggccaag aagctcgagg	1440
ttgccagcgc caacagggt gagcatctca agtactattc cccgcctgcc gacgggaact	1500
gtggttggca ctgcatttcc gccattacca accggatggg gaattccaaa tttgaaacca	1560
ctcttcccga gagagtgaga ccttcagatg actgggctac tgacgaggat cttgtgaata	1620
ccatccaaat cctcaggctc cccgcggcct tggacaggaa cgggtgcttg gctggcgcca	1680
agtacgtgct caagctggaa ggtgagcact ggaccgtctc tgtgaccctt gggatgacct	1740
cttctttgct ccccttgaa tgtgttcagg gttgttgtga gcataagagc ggtcttggtt	1800
tcccagacgt ggtcgaagtt tccggatttg accctgcctg tcttgaccga cttgctgaga	1860
taatgcactt gcctagcagt gtcaccccag ctgctctggc cgagatgtcc gacgacttca	1920
atcgtctggc ttccccggcc gccactgtgt ggactgttcc gcaattcttt gcccgccaca	1980
gaggaggaga gcacctgac cagggtgtgct tagggaaaat tatcaacctt tgtcagggtga	2040
ttgaggaatg ctgctgttcc cggaacaaag ccaaccgggc taccceggaa gaggttgagg	2100
caaaagttga ccagtacctc cgtgggtgcag caagccttgg agaagcttg gccaaagcttg	2160
agagggtcgc cccgccgagc gcgacggaca cctcctttga ttggaatgtt gtgcttctcg	2220
gggttgagac ggcgaaatcag acaaccaaac agctccatgt caaccagtgc cgcgctctgg	2280
ttcctgtcgt gactcaagag cctttggaca gagactcggc ccctctgacc gccttctcgc	2340
tgtccaattg ctactaccct gcacaagggt acgagggtcc tcaccgtgag aggtctaaact	2400
ccttgctctc taagttggag ggggttgttc gtgaggaata tgggctcacg ccaactggac	2460
ctggcccgcg acccgactg ccgaacgggc tcgacgagct taaagaccag atggaggagg	2520
atctgctgaa attagtcaac gcccaggcaa cttcagaaat gatggccttg gcagccgagc	2580
aggttgatct aaaagcttgg gtcaaaaatt acccacgggt gacaccgcca cccctccac	2640
caagagttca gcctcgaaaa acgaagtctg tcaagagctt gctagagaac aagcctgtcc	2700
ctgctccgcg caggaaggtc agatctgatt gtggcagccc gattttgatg ggcgacaatg	2760
ttcctaacgg ttgggaagat tcgactgttg gtggtccctt tgatctttcg gcaccatccg	2820
agccgatgac acctctgagt gagcctgtac ttatttccag gccagtgaca tctttgagtg	2880
tgccggcccc agttcctgca ccgcgtagag ctgtgtcccg accgatgacg ccctcgagtg	2940
agccaatttt tgtgtctgca ctgcgacaca aatttcagca ggtggaaaaa gcaaatctgg	3000

-continued

cggcagcagc	gccgatgtgc	caggacgaac	ccttagattt	gtctgcatcc	tcacagactg	3060
aatatgaggg	ttcccccta	acaccaccgc	agaacgtggg	cattctggag	gtaagggggc	3120
aagaagctga	ggaagtctg	agtgaaatct	cggatattct	gaatgatacc	aacctgcac	3180
ctgtgtcatc	aagcagctcc	ctgtcaagtg	ttaagatcac	acgccccaaa	tactcagctc	3240
aagccattat	cgactcgggc	gggccctgca	gtgggcacct	ccaaaggga	aaagaagcat	3300
gcctccgcac	catgcgtgag	gcttgtgatg	cggccaagct	tagtgaccct	gccacgcagg	3360
aatggctttc	tcgcatgtgg	gataggggtg	acatgctgac	ttggcgcaac	acgtctgctt	3420
accaggcggt	tcgcacctta	gatggcaggt	ttgggtttct	cccaaagatg	atactcgaga	3480
cgcgcgcgc	ctaccctgtg	gggtttgtga	tgttgccctc	cacctctgca	ccttccgtga	3540
gtgcagagag	cgaccttacc	attggttcag	tcgccactga	agatattcca	cgcacctcgc	3600
ggaaaataga	aaataccggt	gagatgatca	accagggacc	cttggcatcc	tctgaggaag	3660
aaccggtata	caaccaacct	gccaaagact	cccggatata	gtcgcggggg	tctgacgaga	3720
gcacagcagc	tccgtccgcg	ggtacaggtg	gcgcgggctt	atttactgat	ttgccacctt	3780
cagacggcgt	agatgcggac	ggtggggggc	cgttgccagc	ggtaagaaag	aaagctgaaa	3840
ggctcttcga	ccaattgagc	cgtcagggtt	ttaacctcgt	ctcccatctc	cctgttttct	3900
tctcacacct	cttcaaactc	gacagtgggt	attctccggg	tgattggggg	tttgcagctt	3960
ttactctatt	ttgcctcttt	ttgtgttaca	gctacccatt	cttcggtttc	gttccctctc	4020
tgggtgtatt	ttctgggtct	tctcggcggt	tgcccatggg	ggtttttggc	tgttggtggg	4080
cttttctgtg	tggcctgttc	aagcctgtgt	ccgaccaggt	cggcactgct	tgtgagtttg	4140
actcgccaga	gtgcaggaac	gtccttcatt	cttttgagct	tctcaaacct	tgggaccctg	4200
ttcgcagcct	tgttgtgggc	cccgctcggt	tcggtcttgc	cattcttggc	aggttactgg	4260
gcggggcacg	ctacatctgg	cattttttgc	ttaggcttgg	cattgttgca	gattgtatct	4320
tggctggagc	ttatgtgctt	tctcaaggta	ggtgtaaaaa	gtgctgggga	tcttgataaa	4380
gaactgctcc	taatgaaatc	gccttcaacg	tgttcccttt	tacacgtgcg	accaggctgt	4440
cactcatcga	cctgtgcgat	cggttttgtg	cgccaacagg	catggacccc	attttcctcg	4500
ccactgggtg	gcgtgggtgc	tggaccggcc	gaagtcccat	tgagcaaccc	tctgaaaaac	4560
ccatcgcggt	cgcacagttg	gatgaaaaga	ggattacggc	tagaactgtg	gtcgtcagc	4620
cttatgatcc	taatcaagcc	gtgaagtgct	tgcgggtgtt	acaggcgggt	ggggcgatgg	4680
tggccgaggg	agtcccaaaa	gtggccaaag	tttctgctat	tccattccga	gccccctttt	4740
ttcccaccgg	agtgaaagtt	gatcccaggt	gcaggatcgt	ggttgacccc	gatactttta	4800
ctacagccct	cgggtctggt	tactctacca	caaacctcgt	ccttggtgtg	ggggactttg	4860
cccagctgaa	tggactaaag	atcaggcaaa	tttccaagcc	ttcgggagga	ggcccacacc	4920
tcatgtctgc	cctgcatgtt	gcctgctcga	tggcgttgca	catgcttgct	ggggtttatg	4980
taacttcagt	ggggtcttgc	ggtgccggca	ccaacgatcc	atgggtgact	aatccgtttg	5040
ccgttctctg	ctacggacca	ggctctctct	gcacgtccag	attgtgcatc	tcccaacatg	5100
gccttaccct	gcccttgaca	gcacttgtgg	cgggattcgg	tcttcaggaa	atcgccctgg	5160
tcgttttgat	tttcgtttcc	atcggaggca	tggctcatag	gttgagttgt	aaggctgata	5220
tgtgtgcat	tttacttgca	atcgccagct	atgtttgggt	accccttacc	tggttgcttt	5280
gtgtgtttcc	ttgttggttg	cgtgggttct	ctttgcaccc	ccttaccatc	ctatgggttg	5340
tgtttttctt	gatttctgta	aatatgcctt	cgggaatctt	ggccgtgggt	ttattggttt	5400

-continued

ctctttggct	tttgggacgt	tatactaaca	ttgtcgggtct	tgtcaccccc	tatgatattc	5460
atcattacac	cagtggcccc	cgcgggtgttg	cgcctttggc	taccgcacca	gatggaacct	5520
acttggctgc	cgtccgccgc	gctgcgttga	ctggtcgcac	catgctgttc	accccgcttc	5580
agcttgggtc	ccttcttgag	ggcgctttca	gaactcgaaa	gccctcactg	aacaccgtea	5640
atgtggttgg	gtcctccatg	ggctctgggtg	gagtgttcac	catcgacggg	aaaattaggt	5700
gcgtgactgc	cgcacatgtc	cttacgggta	attcggctag	ggtttcggga	gtcggcttca	5760
atcaaagtct	tgactttgat	gtgaaagggg	acttcgccat	agctgattgc	ccgaattggc	5820
aaggagctgc	tcccaagacc	caattctgcg	aggacggatg	gactggccgt	gcctattggc	5880
tgacatcctc	tggcgctcga	cccggtgtta	ttgggaatgg	attcgccttc	tgettccaccg	5940
cgtgcggcga	ttccgggtcc	ccagtgatca	ccgaagctgg	tgagattgtc	ggcggtcaca	6000
caggatcaaa	taaacaagga	ggtggcatcg	tcacgcgcgc	ttcaggccag	ttttgtaacg	6060
tggcacccat	caagctgagc	gaattaagtg	aattctttgc	tggacccaag	gtcccgcctc	6120
gtgatgtgaa	ggttggcagc	cacataatta	aagacacgtg	cgaagtacct	tcagatcttt	6180
gcgccttgct	tgctgccaaa	cctgaactgg	agggaggcct	ctccaccgtc	caacttctgt	6240
gtgtgttttt	cctactgtgg	agaatgatgg	gacatgcctg	gacgcccttg	gttgcgtgtg	6300
ggtttttcat	tctgaatgag	gttctcccag	ctgtcctggg	tcggagtgtt	ttctcctttg	6360
ggatgtttgt	gctatcttgg	ctcacaccat	ggtctgcgca	agttctgatg	atcaggcttc	6420
taacagcagc	tcttaacagg	aacagatggg	cacttgccct	ttacagcctt	ggtgcgggtga	6480
ccggttttgt	cgcagatctt	gcggtaactc	aagggcaccc	gttgcaggca	gtaatgaatt	6540
tgagcaccta	tgccttcctg	cctcggatga	tggttgtgac	ctcaccagtc	ccagtgattg	6600
cgtgtggtgt	tgtgcaccta	cttgccatca	ttttgtactt	gttcaagtac	cgcggcctgc	6660
acaatgttct	tgttggtgat	ggagcgtttt	ctgcagcttt	cttcttgcca	tactttgccg	6720
agggaaagtt	gaggggaagg	gtgtcgcaat	cctgcggaat	gaatcatgag	tcattgactg	6780
gtgccctcgc	tatgagactc	aatgacgagg	acttggaact	ccttacgaaa	tggactgatt	6840
ttaagtgcct	tgtttctgcg	tccaacatga	ggaatgcagc	aggccaattc	atcgaggctg	6900
cctatgcaaa	agcacttaga	attgaacttg	cccagttggg	gcaggttgat	aaggttcgag	6960
gtactttggc	caagcttgag	gcttttgctg	ataccgtggc	accccaactc	tcgcccgggtg	7020
acattgttgt	tgctcttggc	catacgccctg	ttggcagcat	cttcgaccta	aaggttgggtg	7080
gtaccaagca	tactctccaa	gtcattgaga	ccagagtcct	tgccgggtcc	aaaatgaccg	7140
tggcgcgcgt	cgttgaccca	acccccacgc	ccccaccgc	acccgtgccc	atccccctcc	7200
caccgaaagt	tctagagaat	ggtcccaacg	cctgggggga	tggggaccgt	ttgaataaga	7260
agaagaggcg	taggatggaa	accgtcggca	tctttgtcat	gggtgggaag	aagtaccaga	7320
aattttggga	caagaattcc	ggtgatgtgt	tttacgagga	ggtccatgac	aacacagatg	7380
cgtgggagtg	cctcagagtt	ggtgaccctg	ccgactttaa	ccctgagaag	ggaactctgt	7440
gtgggcatac	tactattgaa	gataaggatt	acaaagtcta	cgcctcccca	tctggcaaga	7500
agttcctggg	ccccgtcaac	ccagagagcg	gaagagccca	atgggaagct	gcaaagcttt	7560
ccgtggagca	ggcccttggc	atgatgaatg	tcgacgggtga	actgacggcc	aaagaagtgg	7620
agaaaactgaa	aagaataatt	gacaaaacttc	agggcctgac	taaggagcag	tgtttaaact	7680
gctagccgcc	agcggcttga	cccgtgtgtg	tcgcggcgcc	ttggttggtta	ctgagacagc	7740

-continued

ggtaaaaata	gtcaaatttc	acaaccggac	tttcacccta	gggcctgtga	atttaaaagt	7800
ggccagtgag	gttgagctga	aagacgcggt	cgagcacaca	caacaccccg	ttgcaagacc	7860
ggttgacggt	ggtgttgtgc	tectgcgttc	cgagtttctt	tcgtttatag	atgtctgat	7920
ctccggtgct	gacgcatttc	ctaagttact	cgctcgtcac	gggcggggga	acactgggat	7980
cgatggcacg	ctttgggact	ttgaggccga	ggccacccaa	gaggaaattg	cgctcagtgc	8040
gcaaataata	caggcttgtg	acattaggcg	cggtgacgca	cctgaaattg	gtctccctta	8100
caagctgtac	cctgttaggg	gcaaccctga	gcgggtaaaa	ggagttttac	agaatacaag	8160
gtttggagac	ataccttaca	aaacccccag	tgacactggg	agcccagtgc	acgcggctgc	8220
ctgcctcacg	cccaatgcca	ctccggtgac	tgatggggcg	tccttcttgg	ctactaccat	8280
gccctccggt	tttgaattgt	atgtaccgac	cattccagcg	tctgtccttg	attatcttga	8340
ctctaggcct	gactgcccc	aacagttgac	agagcacggc	tgtgaggatg	ccgcattgag	8400
agacctctcc	aagtatgact	tgtccacca	aggttttgtt	ttacctgggg	ttcttcgcct	8460
tgtgcgtaag	tacctgtttg	cccacgtggg	taagtgcggg	cccgttcctc	ggccttccac	8520
ttacctgcc	aagaattcta	tggttggaat	aaatgggaac	aggtttccaa	ccaaggacat	8580
tcagagcgtc	cccgaaatcg	acgttctgtg	cgcacaggcc	gtgcgagaaa	actggcaaac	8640
tgttacccct	tgtaccctca	agaaacagta	ttgtgggaag	aagaagacta	ggacaatact	8700
cggcaccaat	aatttcattg	cgttgggcca	ccgggcagcg	ttgagtgggt	tcaccagggt	8760
cttcatgaaa	aaggcgttta	actcgcccat	cgccctcggt	aaaaacaaat	ttaaggagct	8820
acagactccg	atcttaggca	ggtgccttga	agctgatctt	gcacctgtgt	atcgatccac	8880
acctgcaatt	gtccgctggt	ttgcgcgcaa	ccttctttat	gaacttgcct	gtgctgaaga	8940
gcacctaccg	tcgtacgtgc	tgaactgctg	ccatgacctt	ttggtcacgc	agtcgggcgc	9000
agtgactaag	aggggtggcc	tgtcgtcttg	cgacccgatc	acttctgtgt	ctaacaccat	9060
ttacagcttg	gtgatatatg	cacagcacat	ggtgcttagt	tactttaaaa	gtggtcacc	9120
tcattggcctt	ctgttctctc	aagaccagct	gaagtctgag	gacatgctca	aagtccaacc	9180
cctgatcgtc	tattcgagcg	acctcggtgt	gtatgcggaa	tctccaccca	tgcggaacta	9240
ccactgggtgg	gtcgaaacatc	tgaatttgat	gctgggtttt	cagacggacc	caaagaagac	9300
agccataacg	gactcgccat	catttctagg	ctgtaggata	ataaatggac	gccagctagt	9360
ccccaaccgt	gacaggatcc	tcggggccct	cgcttaccat	atgaaggcaa	gcaatgtttc	9420
tgaatactac	gccgcgggcg	ctgcaatact	catggacagc	tgtgcttgtt	tagagtatga	9480
tcctgaatgg	tttgaagagc	ttgtgggttg	gatagcgag	tgcgcccga	aggacggcta	9540
cagctttccc	ggcccgccgt	tcttcttgtc	catgtgggaa	aaactcagat	ccaatcatga	9600
ggggaagaag	tccagaatgt	gcgggtattg	cggggccccg	gctccgtacg	ccactgcctg	9660
tggcctcgac	gtctgtatct	accacacca	cttccaccag	cattgtccag	tcacaatctg	9720
gtgtggccac	ccggctggtt	ctgggttctt	tagtgagtgc	aaaccccccc	tagggaaagg	9780
cacaagccct	ctagatgagg	tgttagaaca	agtcccgtat	aagcctccac	ggactgtaat	9840
catgcatgtg	gagcagggtc	tcacccctct	tgacccaggc	agataccaga	ctcgccgcgg	9900
attagtctcc	gttaggcgtg	gcacagagg	aaatgaagtt	gacctaccag	acggtgatta	9960
tgctagcacc	gccctactcc	ccacttgtaa	agagatcaac	atggtegtgt	tcgcctctaa	10020
tgtgttgccg	agcaggttca	tcacgggtcc	gcccgggtgt	gggaaaacat	actggctcct	10080
tcagcaggtc	caggatggtg	atgtcattta	cacaccgact	caccagacca	tgctcgacat	10140

-continued

gattagggct	ttggggacgt	gccgggtcaa	cgtcccagca	ggtacaacgc	tgcaattccc	10200
tgccccctcc	cgtaccggcc	cgtgggttcg	catcctagcc	ggcggttggt	gtcctggtaa	10260
gaattccttc	ttggatgaag	cagcgtattg	taatcacctt	gatgtcttga	ggctccttag	10320
caaaaccacc	ctcacctgtc	tgggagactt	caaacaactc	caccagtggt	gttttgattc	10380
tcattgctat	gtttttgaca	tcatgcctca	gaccagttg	aagaccatct	ggagattcgg	10440
acagaacatc	tgtgatgcca	tccaaccaga	ttacagggac	aaacttgtgt	ccatgggtcaa	10500
cacaaccctg	gtaacctacg	tggaaaaacc	tgtcaagtat	gggcaagtcc	tcacccctta	10560
ccacagggac	cgagaggacg	gcgccatcac	aattgactcc	agtcaaggcg	ccacatttga	10620
tgtggtcaca	ctgcatttgc	ccactaaaga	ttcactcaac	aggcaaagag	cccttgttgc	10680
tatcaccagg	gcaagacatg	ctatctttgt	gtatgaccca	cacaggcaat	tgcaagagcat	10740
gtttgatctt	cctgcgaagg	gcacaccctg	caacctcgca	gtgcaccgtg	atgagcagct	10800
gacgtactg	gatagaaata	ataaagaatg	cacagttgct	caggctctag	gcaacggaga	10860
taaatttagg	gccaccgaca	agcgcgttgt	agattctctc	cgcgccattt	gtgctgatct	10920
ggaagggctg	agctctccgc	tccccagggt	cgcacacaac	ttgggatttt	atttctcacc	10980
tgatttgaca	cagtttgcta	aactcccggg	agaccttgca	ccccactggc	ccgtgggtgac	11040
aacccagaac	aatgaaaagt	ggccggatcg	gctggttgcc	agccttcgcc	ctgtccataa	11100
gtatagccgt	gcgtgcattg	gtgccggcta	tatggtgggc	ccctcggtgt	ttctaggcac	11160
ccctgggggc	gtgtcatact	acctcacaaa	atttgtcaag	ggcgaggctc	aagtgcttcc	11220
ggagacagtc	ttcagcaccg	gccgaattga	ggtggattgc	cgggagtatc	ttgatgacag	11280
ggagcgagaa	gttgctgagt	ccctcccaca	tgcttcatt	ggcgacgtca	aaggcaccac	11340
cgttggggga	tgtcatcatg	tcacctccaa	ataccttcgg	cgttccttc	ccaaggaatc	11400
agtcgcggta	gtcggggttt	cgagccccgg	gaaagccgca	aaagcagtgt	gcacattgac	11460
ggatgtgtac	ctcccagacc	ttgaggccta	cctccacca	gagactcagt	ccaagtgtctg	11520
gaaagtatat	ttggacttca	aggaagttcg	actgatggtc	tggaaagaca	agacggccta	11580
tttccaactt	gaaggccgct	atttcacctg	gtatcagctt	gcaagctacg	cctcgtacat	11640
ccgtgttcc	gtcaactcca	cgggtgtatct	ggacccctgc	atgggccttg	ccctttgcaa	11700
cagaagagtt	gtcgggtcca	cccatggggg	agctgacctc	gcagtcaccc	cttatgatta	11760
cggtgctaaa	atcatcttgt	ctagcgctta	ccatggtgaa	atgcctcctg	gatacaagat	11820
tctggcgtgc	gcgaggttct	cgtcgcacga	cccagtcga	tacaaacaca	cctgggggtt	11880
tgaatcggt	acagcgtatc	tgtatgagtt	caccggaaac	ggtgaggact	gggaggatta	11940
caatgatgcg	tttcgtgcgc	gccagaaagg	gaaaatttat	aaggccactg	ctaccagcat	12000
gaagttttat	tttcccccg	gccccgtcat	tgaaccaact	ttaggcctga	attgaaatga	12060
aatggggct	atacaaagcc	tcttcgacaa	aattggccag	ctttttgtgg	atgctttcac	12120
ggaatttttg	gtgtccattg	ttgatatcat	catatttttg	gccattttgt	ttggcttcac	12180
catcgccggt	tggctgggtg	tcttttgcat	cagattgggt	tgctccgcgg	tattccgtgc	12240
gcgccctgcc	attcacctg	agcaattaca	gaagatccta	tgaggccttt	ctttctcagt	12300
gccgggtgga	cattcccacc	tggggggtaa	aacacccttt	ggggatgttt	tggcaccata	12360
aggtgtcaac	cctgattgat	gaaatgggtg	cgcgtcgaat	gtaccgcatc	atggaaaaag	12420
cagggcaagc	tgctggaaa	caggtgggtga	gcgaggctac	gctgtctcgc	attagtagtt	12480

-continued

tggatgtggt	ggctcatttt	caacatcttg	ccgccattga	agccgagacc	tgtaaatt	12540
tggcttctcg	actgcccattg	ctacacaacc	tgcgcatgac	aggggtcaa	gtaaccatag	12600
tgtataatag	cacttttaaat	caggtgtttg	ctatttttcc	aacctctggt	tcccggccaa	12660
agcttcatga	ttttcagcaa	tggctaata	ctgtacattc	ctccatattt	tcctctgttg	12720
cagcttcttg	tactcttttt	gttgtgctgt	ggttgccggg	tccaatgcta	cgtactgttt	12780
ttggtttccg	ctggttagg	gcaatttttc	tttcgaactc	atgggtgaatt	acacgggtgtg	12840
tccaccttgc	ctcaccgcg	aagcagccgc	tgaggctcct	gaacctcggt	ggtctctttg	12900
gtgcaggata	gggcatgacc	gatgtgggga	ggacgatcac	gacgaactgg	ggttcatggt	12960
tccgcctggc	ctctccagcg	aaagccactt	gaccagtgtt	tacgcctggt	tggcgcttcc	13020
gtccttcagc	tacacggccc	agttccatcc	cgagatattt	gggataggga	acgtgagtga	13080
agtttatggt	gacatcaagc	accaattcat	ctgcgcggtt	catgacgggc	agaacaccac	13140
cttgctcgc	catgacaata	tttcagccgt	atttcagacc	tactatcaac	atcagggtcga	13200
cggcggcaat	tggtttcacc	tagaatggct	gcgtcccttc	ttttcctctt	ggttggtttt	13260
aaatgtttcg	tggtttctca	ggcgctcgcc	tgcaagccat	gtttcagttc	gagtctttca	13320
gacatcaaaa	ccaacactac	cgcagcatca	ggctttgttg	tcctccagga	catcagctgc	13380
cttaggcattg	gcgactcgtc	ctttccgacg	attcgcaaaa	gctctcaatg	ccgcacggcg	13440
atagggacac	ctgtgtatat	caccatcaca	gccaatgtga	cagatgagaa	ttacttacat	13500
tcttctgac	tcctcatgct	ttcttcttgc	cttttctatg	cttctgagat	gagtgaagaa	13560
ggattcaagg	tggatatttg	caatgtgtca	ggcatcgctg	ctgtgtgtgt	caactttacc	13620
agctacgtcc	aacatgtcaa	agagtttacc	caacgctcct	tgggtggtcga	tcattgtgcg	13680
ctgcttcatt	tcattgacac	tgagaccatg	aggtgggcaa	ccgttttagc	ctgtcttttt	13740
gccatcctac	tggcaatttg	aatgttcaag	tatgttgggg	aaatgcttga	ccgcgggctg	13800
ttgctcgcga	ttgctttctt	tgtggtgtat	cgtgccgttc	tggtttgctg	tgtcgggcaa	13860
cgccaacagc	agcagcagct	ctcattttca	gttgatttat	aacttgacgc	tatgtgagct	13920
gaatggcaca	gattggctgg	cagaaaaatt	tgattgggca	gtggagactt	ttgtcatctt	13980
tcccggtgtg	actcacattg	tttcctatgg	tgactcacc	accagccatt	tccttgacac	14040
agttgggtctg	gttactgtgt	ccaccgcccg	gttttatcac	gggcgggtatg	tcttgagtag	14100
catctacgcg	gtctgtgctc	tggctgcgtt	gatttgcttc	gttattaggc	ttgcgaagaa	14160
ctgcatgtcc	tggcgctact	cttgtagcag	atataccaac	ttccttcttg	acaactaagg	14220
cagactctat	cgttggcggt	cgcgcgttat	catagaaaaa	gggggtaagg	ttgaggtcga	14280
aggtcatctg	atcgacctca	aaagagttgt	gcttgatggt	tccgtggcaa	cccctttaac	14340
cagagtttca	gcggaacaat	ggggtcgtct	ctagacgact	tttgccatga	tagcacggct	14400
ccacaaaagg	tgcttttggc	gttttccatt	acctacacgc	cagtaatgat	atatgctcta	14460
aaggtaagtc	gcggccgact	gctagggctt	ctgcaccttt	tgatctttct	gaattgtgct	14520
tttaccttcg	ggtacatgac	attcgccgac	tttcagagca	caaatagggt	cgcgctcgct	14580
atgggagcag	tagttgcact	tctttggggg	gtgtactcag	ccatagaaac	ctggaaattc	14640
atcacctcca	gatgccgttt	gtgcttgcta	ggccgcaagt	acattctggc	ccctgcccac	14700
cacgtcgaaa	gtgcgcggg	ctttcatccg	attgcggcaa	atgataacca	cgcatttgtc	14760
gtccggcgtc	ccggctccat	tacggttaac	ggcacattgg	tgccggggtt	gaaaagcctc	14820
gtgttggtg	gcagaaaagc	tgttaaagc	ggagtggtaa	accttgctca	atatgccaaa	14880

-continued

taacaacggc	aagcagcaaa	agaaaaagaa	ggggaatggc	cagccagtca	accagctgtg	14940
ccagatgctg	ggtaaaatca	tcgcccagca	aaaccagtec	agaggcaagg	gaccggggcaa	15000
gaaaagtaag	aagaaaaacc	cggagaagcc	ccattttcct	ctagcgaccg	aagatgacgt	15060
caggcatcac	ttcacccttg	gtgagcggca	attgtgtctg	tcgtcgatcc	agactgcctt	15120
taaccagggc	gctggaactt	gtaccctgtc	agattcaggg	aggataagtt	acactgtgga	15180
gtttagtttg	ccgacgcata	atactgtgcg	cctgatccgc	gtcacagcat	caccctcagc	15240
atgatgggct	ggcattcttt	aggcacctca	gtgtcagaat	tggaagaatg	tgtgggtggat	15300
ggcactgatt	gacattgtgc	ctctaagtca	cctattcaat	tagggcgacc	gtgtgggggt	15360
aaaatttaat	tggcgagaac	catgcgcccg	caattaaaaa	aaaaaaaaaa	aaaaaaaaaa	15420
aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa				15450

<210> SEQ ID NO 2
 <211> LENGTH: 15444
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: P129-PK-d43/44 passage 17 complete genome
 (15,444 nt) 2 aa deleted infectious clone

 <400> SEQUENCE: 2

atgacgtata	ggtgttggct	ctatgccacg	gcatttgtat	tgtcaggagc	tgtgaccatt	60
ggcacagccc	aaaacttgct	gcacggaaaa	cgccttctg	tgacagcctt	cttcagggga	120
gcttaggggt	ctgtccctag	caccttgctt	ctggagttgc	actgctttac	ggtctctcca	180
cccctttaac	catgtctggg	atacttgatc	ggtgcacgtg	cacccccaat	gccaggtgt	240
ttatggcgga	gggccaagtc	tactgcacac	gatgtctcag	tgacaggtct	ctccttctc	300
tgaatctcca	agttcctgag	cttgggggtg	tgggcctatt	ttataggccc	gaagagccac	360
tccggtggac	gttgccacgt	gcattcccca	ctgtcgagtg	ctccctgccc	ggggcctgct	420
ggctttctgc	gatctttcca	attgcacgaa	tgaccagtgg	aaacctgaac	tttcaacaaa	480
gaatggtgcg	ggttgacagc	gagatttaca	gagccggcca	actcaccctt	gcagttttga	540
aggctctaca	agtttatgaa	cgggggtgtc	gctggtagcc	cattgtcgga	cctgtccctg	600
gagtggccgt	ttacgccaac	tccctacatg	tgagtgacaa	acctttcccg	ggagcaactc	660
atgtgttaac	caacctaccg	ctcccgcaga	ggccaagcc	tgaagacttt	tgcccttttg	720
agtgtgctat	ggctaacgtc	tatgacattg	gccataacgc	cgtcatgtat	gtggccagag	780
ggaaagtctc	ctgggcccct	cgtggcgggg	atgaagtga	atttgaaacc	gtccccgaag	840
agttgaagtt	gattgcgaac	cgactccaca	tctccttccc	gccccaccac	gcagtgga	900
tgtctgagtt	tgccttcata	gcccctggga	gtggtgtctc	cttgccgggtc	gagcaccaac	960
acggctgcct	tcccgtgat	actgtccctg	atgggaactg	ctgggtgtac	ttgtttgact	1020
tgtcccacc	ggaagttcag	aataaagaaa	ttcgccgtgc	taaccaattt	ggctatcaaa	1080
ccaagcatgg	tgtccatggc	aagtacctac	agcggaggct	gcaagttaat	ggtctccgag	1140
cagtgactga	tacagatgga	cctattgtcg	tacagtactt	ctctgttagg	gagagttgga	1200
tccgccactt	cagattggcg	gaagaacctc	gcctccctgg	gtttgaagac	ctcctcagaa	1260
taagggtaga	gcctaatacg	tcgccattgg	gtggcaaggg	tgaaaaaatc	ttccggtttg	1320
gcagtcacaa	gtggtacggg	gctggaaaga	gagcaaggag	agcacgctct	ggtgcgactg	1380
ccacggctgc	tcaccgcgct	ttgcccgtc	gcgaagccca	gcaggccaag	aagctcgagg	1440

-continued

ttgccagcgc	caacagggct	gagcatctca	agtactattc	cccgcctgcc	gacgggaact	1500
gtggttggca	ctgcatttcc	gccattacca	accggatggg	gaattccaaa	tttgaaacca	1560
ctcttcccga	gagagtgaga	ccttcagatg	actgggctac	tgacgaggat	cttgtgaata	1620
ccatccaaat	cctcaggctc	cccgcggcct	tggacaggaa	cggtgcttgt	gctggcgcca	1680
agtacgtgct	caagctggaa	ggtgagcact	ggaccgtctc	tgtgaccctc	gggatgaccc	1740
cttctttgct	cccccttgaa	tgtgttcagg	gttgttgtga	gcataagagc	ggtcttggtt	1800
tcccagacgt	ggtcgaagtt	tccggatttg	accctgcctg	tcttgaccga	cttgctgaga	1860
taatgcactt	gcctagcagt	gtcatcccag	ctgctctggc	cgagatgtcc	gacgacttca	1920
atcgtctggc	ttccccggcc	gccactgtgt	ggactgtttc	gcaattcttt	gcccggcaca	1980
gaggaggaga	gcacctgac	cagggtgtgct	tagggaaaat	tatcaacctt	tgtcagggtga	2040
ttgaggaatg	ctgctgttcc	cggaaacaaag	ccaaccgggc	taccceggaa	gaggttgccg	2100
caaaagttga	ccagtacctc	cgtgggtgcag	caagccttgg	agaatgcttg	gccaagcttg	2160
agagggctcg	cccgcggagc	gcgacggaca	cctcctttga	ttggaatgtt	gtgcttctcg	2220
gggttgagac	ggcgaatcag	acaaccaaac	agctccatgt	caaccagtgc	cgcgctctgg	2280
ttcctgtcgt	gactcaagag	cctttggaca	gagactcggg	ccctctgacc	gccttctcgc	2340
tgtccaattg	ctactaccct	gcacaagggt	acgagggtcc	tcaccgtgag	aggctaaact	2400
ccttgctctc	taagttggag	ggggttgttc	gtgaggaata	tgggctcacg	ccaactggac	2460
ctggcccgcg	accgcactg	cgaacgggc	tcgacgagct	taaagaccag	atggaggagg	2520
atctgctgaa	attagtcaac	gcccaggcaa	cttcagaaat	gatggcctgg	gcagccgagc	2580
aggttgatct	aaaagcttgg	gtcaaaaatt	acccacgggt	gacaccgcca	ccccctccac	2640
caagagttca	gcctcgaaaa	acgaagtctg	tcaagagctt	gctagagaac	aagcctgtcc	2700
ctgctccgcg	caggaaggtc	agatctgatt	gtggcagccc	gattttgatg	ggcgacaatg	2760
ttcctaacgg	ttgggaagat	tcgactgttg	gtggtccctc	tgatctttcg	gcaccatccg	2820
agccgatgac	acctctgagt	gagcctgtac	ttatttccag	gccagtgaca	tctttgagtg	2880
tgcggccccc	agttcctgca	ccgcgtagag	ctgtgtcccg	accgatgacg	ccctcgagtg	2940
agccaatttt	tgtgtctgca	ctgcgacaca	aatttcagca	ggtggaaaaa	gcaaactctgg	3000
cggcagcagc	gccgatgtgc	caggacgaac	ccttagattt	gtctgcatcc	tcacagactg	3060
aatatgaggc	ttcccccta	acaccaccgc	agaacgtggg	cattctggag	gtaagggggc	3120
aagaagctga	ggaagtctg	agtgaatctc	cggatattct	gaatgatacc	aacctgcac	3180
ctgtgtcatc	aagcagctcc	ctgtcaagtg	ttaagatcac	acgccccaaa	tactcagctc	3240
aagccattat	cgactcgggc	gggccttgca	gtgggcacct	ccaaagggaa	aaagaagcat	3300
gcctccgcac	catgcgtgag	gcttgtgatg	cggccaagct	tagtgaccct	gccacgcagg	3360
aatggctttc	tcgcatgtgg	gataggggtg	acatgctgac	ttggcgcaac	acgtctgctt	3420
accaggcggt	tcgcacctta	gatggcaggt	ttgggtttct	cccaaagatg	atactcgaga	3480
cgcgcgcgcc	ctaccctgtg	gggtttgtga	tgttgccctc	cacccttgca	ccttccgtga	3540
gtgcagagag	cgaccttacc	attggttcag	tcgccactga	agatattcca	cgcacctcgc	3600
ggaaaataga	aaataccggt	gagatgatca	accagggacc	cttggcatcc	tctgaggaag	3660
aaccggtata	caaccaacct	gcccagact	cccggatata	gtcgcggggg	tctgacgaga	3720
gcacagcagc	tccgtccgcg	ggtacaggtg	gcgccggctt	atttactgat	ttgccacctt	3780

-continued

cagacggcgt	agatgcggac	ggtggggggc	cgttgccagac	ggtaagaaag	aaagctgaaa	3840
ggctcttcga	ccaattgagc	cgtcagggtt	ttaacctcgt	ctcccatctc	cctgttttct	3900
tctcacacct	cttcaaactc	gacagtgggt	attctccggg	tgattggggg	tttgcagctt	3960
ttactctatt	ttgcctcttt	ttgtgttaca	gctacccatt	cttcggtttc	gttccccctc	4020
tgggtgtatt	ttctgggtct	tctcggcgtg	tgcgcatggg	ggtttttggc	tgctggctgg	4080
cttttgctgt	tggcctgttc	aagcctgtgt	ccgacccagt	cggcactgct	tgtgagtttg	4140
actcgccaga	gtgcaggaac	gtccttcatt	cttttgagct	tctcaaacct	tgggaccctg	4200
ttcgcagcct	tgttgtgggc	cccgctcggtc	tcggtcttgc	cattcttggc	aggttactgg	4260
gcggggcacg	ctacatctgg	cattttttgc	ttaggcttgg	cattgttgca	gattgtatct	4320
tggctggagc	ttatgtgctt	tctcaaggta	ggtgtaaaaa	gtgctgggga	tcttgataaa	4380
gaactgctcc	taatgaaatc	gccttcaacg	tgttcccttt	tacacgtgcg	accaggctcg	4440
cactcatcga	cctgtgcgat	cggttttgtg	cgccaacagg	catggacccc	attttcctcg	4500
ccactgggtg	gcgtgggtgc	tggaccggcc	gaagtcccat	tgagcaaccc	tctgaaaaac	4560
ccatcgcggt	cggccagttg	gatgaaaaga	ggattacggc	tagaactgtg	gtcgtcagc	4620
cttatgatcc	taatcaagcc	gtgaagtgct	tgcgggtggt	acaggcgggt	ggggcgatgg	4680
tggccgaggc	agtcccaaaa	gtggccaaag	tttctgctat	tccattccga	gccccctttt	4740
ttcccaccgg	agtgaaagtt	gatcccagat	gcaggatcgt	ggttgacccc	gatactttta	4800
ctacagccct	ccggtctggt	tactctacca	caaacctcgt	ccttggtgtg	ggggactttg	4860
cccagctgaa	tggactaaag	atcaggcaaa	tttccaagcc	ttcgggagga	ggcccacacc	4920
tcatgtctgc	cctgcatggt	gcctgctcga	tggcgttgca	catgcttgct	ggggtttatg	4980
taacttcagt	ggggtcttgc	ggtgccggca	ccaacgatcc	atggtgcact	aatccgtttg	5040
ccgttcctgg	ctacggacca	ggctctctct	gcacgtccag	attgtgcac	tcccaacatg	5100
gccttaccct	gcccttgaca	gcacttggtg	cgggatccgg	tcttcaggaa	atcgcttggt	5160
tcgttttgat	tttcgtttcc	atcggaggca	tggctcatag	gttgagttgt	aaggctgata	5220
tgctgtgcat	tttacttgca	atcgccagct	atgtttgggt	acccttacc	tggttgcttt	5280
gtgtgtttcc	ttgttggttg	cgtcgggtct	ctttgcaccc	ccttaccatc	ctatgggttg	5340
tgtttttctt	gatttctgta	aatatgcctt	cgggaatctt	ggcgtgggtg	ttattggttt	5400
ctctttggct	tttgggacgt	tatactaaca	ttgtcgggtc	tgtcaccccc	tatgatattc	5460
atcattacac	cagtggcccc	cggggtggtg	ccgccttggc	taccgcacca	gatggaacct	5520
acttggtgc	cgtccgccgc	gctgcgttga	ctggtcgcac	catgctgttc	accccgctct	5580
agcttgggtc	ccttcttgag	ggcgctttca	gaactcgaaa	gccctcactg	aacaccgtca	5640
atgtgggttg	gtcctccatg	ggctctgggt	gagtgttcac	catcgacggg	aaaattaggt	5700
gcgtgactgc	cgcacatgtc	cttacgggta	attcggctag	ggtttccgga	gtcggcttca	5760
atcaaatgct	tgactttgat	gtgaaagggg	acttcgccat	agctgattgc	ccgaattggc	5820
aaggagctgc	tcccaagacc	caattctgcg	aggacggatg	gactggccgt	gcctattggc	5880
tgacatcctc	tggcgtcgaa	cccgggtgta	ttgggaatgg	attcgccttc	tgcttcaccg	5940
cgtgcggcga	ttccgggtcc	ccagtgatca	ccgaagctgg	tgagattgtc	ggcggtcaca	6000
caggatcaaa	taaacaagga	ggtggcatcg	tcacgcgccc	ttcaggccag	ttttgtaacg	6060
tggcaccat	caagctgagc	gaattaagtg	aattctttgc	tggacccaag	gtcccgtctg	6120
gtgatgtgaa	ggttggcagc	cacataatta	aagacacgtg	cgaagtacct	tcagatcttt	6180

-continued

gcgccttgct	tgctgccaaa	cctgaactgg	agggaggcct	ctccaccgtc	caacttctgt	6240
gtgtgttttt	cctactgtgg	agaatgatgg	gacatgcctg	gacgcccttg	gttgetgtgg	6300
ggtttttcat	tctgaatgag	gttctcccag	ctgtcctggg	tccgagtggt	ttctcctttg	6360
ggatgtttgt	gctatcttgg	ctcacaccat	ggctcgcgca	agttctgatg	atcaggcttc	6420
taacagcagc	tcttaacagg	aacagatggg	cacttgccct	ttacagcctt	ggcgcggtga	6480
cgggttttgg	cgcagatctt	gcggtaactc	aagggcaccc	gttgcaggca	gtaatgaatt	6540
tgagcaccta	tgccctcctg	cctcggatga	tggttgtagc	ctcaccagtc	ccagtgattg	6600
cgtgtgggtg	tgtgcaccta	cttgccatca	ttttgtactt	gttcaagtac	cgcggcctgc	6660
acaatgttct	tgttggtgat	ggagcgtttt	ctgcagcttt	cttcttgcca	tactttgccg	6720
agggaaagtt	gaggggaagg	gtgtcgcaat	cctgcggaat	gaatcatgag	tcattgactg	6780
gtgccctcgc	tatgagactc	aatgacgagg	acttggaact	ccttacgaaa	tggaactgatt	6840
ttaagtgcct	tgtttctgcg	tccaacatga	ggaatgcagc	aggccaattc	atcgaggctg	6900
cctatgcaaa	agcacttaga	attgaacttg	cccagttggg	gcaggttgat	aaggttcgag	6960
gtactttggc	caagcttgag	gcttttgctg	ataccgtggc	accccaactc	tcgcccgggtg	7020
acattgttgt	tgctcttggc	catacgccctg	ttggcagcat	cttcgaccta	aaggttgggtg	7080
gtaccaagca	tactctccaa	gtcattgaga	ccagagtcct	tgccgggtcc	aaaatgaccg	7140
tggcgcgcgt	cgttgaccca	acccccacgc	ccccaccgcg	acccgtgccc	atccccctcc	7200
caccgaaagt	tctagagaat	ggccccaacg	cctgggggga	tggggaccgt	ttgaataaga	7260
agaagaggcg	taggatggaa	accgtcggca	tctttgtcat	gggtgggaag	aagtaccaga	7320
aattttggga	caagaattcc	ggatgatgtg	tttacgagga	ggcccatgac	aacacagatg	7380
cgtgggagtg	cctcagagtt	ggtagccctg	ccgactttaa	ccctgagaag	ggaactctgt	7440
gtgggcatac	tactattgaa	gataaggatt	acaaagtcta	cgcctcccca	tctggcaaga	7500
agttcctggg	ccccgtcaac	ccagagagcg	gaagagccca	atgggaagct	gcaaagcttt	7560
ccgtggagca	ggcccttggc	atgatgaatg	tcgacgggtg	actgacggcc	aaagaagtgg	7620
agaaactgaa	aagaataatt	gacaaacttc	agggcctgac	taaggagcag	tgtttaaact	7680
gctagccgcc	agcggcttga	cccgtctgtg	tcgcggcggc	ttggttggtt	ctgagacagc	7740
ggtaaaaata	gtcaaatttc	acaaccggac	tttcacccta	gggcctgtga	atttaaaagt	7800
ggccagtgag	gttgagctga	aagacgcggg	cgagcacaac	caacaccccg	ttgcaagacc	7860
ggttgacggg	gggtgtgtgc	tcctgcgttc	cgaggttcct	tcgcttatag	atgtcctgat	7920
ctccgggtgt	gacgcacttc	ctaagttact	cgctcgtcac	gggcggggga	acactgggat	7980
cgatggcacg	ctttgggact	ttgaggccga	ggccacccaa	gaggaaattg	cgtcagtgct	8040
gcaaataata	caggcttgtg	acattaggcg	cggtagcgca	cctgaaattg	gtctccctta	8100
caagctgtac	cctgttaggg	gcaaccctga	gcgggtaaaa	ggagttttac	agaatacaag	8160
gtttggagac	ataccttaca	aaacccccag	tgacactggg	agcccagtgc	acgcggctgc	8220
ctgcctcacg	cccaatgcca	ctccgggtgac	tgatgggcgc	tccgtcttgg	ctactaccat	8280
gccctccggg	tttgaattgt	atgtaccgac	cattccagcg	tctgtccttg	attatcttga	8340
ctctaggcct	gactgcccc	aacagttgac	agagcacggc	tgtgaggatg	ccgcattgag	8400
agacctctcc	aagtatgact	tgtccacca	aggttttggt	ttacctgggg	ttcttcgcct	8460
tgtgcgtaag	tacctgtttg	cccacgtggg	taagtgcgcc	cccgttcac	ggccttcac	8520

-continued

ttaccctgcc	aagaattcta	tggctggaat	aaatgggaac	aggtttccaa	ccaaggacat	8580
tcagagcgtc	cccgaaatcg	acgttctgtg	cgcacaggcc	gtgcgagaaa	actggcaaac	8640
tgttaccctt	tgtaccctca	agaaacagta	ttgtgggaag	aagaagacta	ggacaatact	8700
cggcaccaat	aatttcattg	cgttggccca	ccgggcagcg	ttgagtgggtg	tcaccaggg	8760
cttcatgaaa	aaggcgttta	actcgcccat	cgcctcggtg	aaaaacaaat	ttaaggagct	8820
acagactccg	atcttaggca	ggtgccttga	agctgatctt	gcacctctgtg	atcgatccac	8880
acctgcaatt	gtccgctggt	ttgccgcca	ccttctttat	gaacttgcct	gtgctgaaga	8940
gcacctaccg	tcgtacgtgc	tgaactgctg	ccatgacctt	ttggtcacgc	agtcggcgcg	9000
agtgactaag	aggggtggcc	tgtcgtctgg	cgcacagctc	acttctgtgt	ctaacaccat	9060
ttacagcttg	gtgatatatg	cacagcacat	ggtgcttagt	tactttaaaa	gtggtcaccc	9120
tcattggcctt	ctgttctctc	aagaccagct	gaagtctcag	gacatgctca	aagtccaacc	9180
cctgatcgtc	tattcgagcg	acctcgctgt	gtatgccgaa	tctccaccca	tgcggaacta	9240
ccactgggtg	gtcgaacatc	tgaatttgat	gctgggtttt	cagacggacc	caaagaagac	9300
agccataacg	gactcgccat	catttctagg	ctgtaggata	ataaatggac	gccagctagt	9360
ccccaaccgt	gacaggatcc	tcggcgccct	cgttaccat	atgaaggcaa	gcaatgtttc	9420
tgaatactac	gccgcgcgcg	ctgcaatact	catggacagc	tgtgcttgtt	tagagtatga	9480
tcctgaatgg	tttgaagagc	ttgtgggttg	gatagcgcag	tgcgcccgcg	aggacggcta	9540
cagctttccc	ggcccgccgt	tcttcttctc	catgtgggaa	aaactcagat	ccaatcatga	9600
ggggaagaag	tccagaatgt	gcgggtattg	cggggccccg	gctccgtacg	ccactgcctg	9660
tggcctcgac	gtctgtatct	accacacca	cttccaccag	cattgtccag	tcacaatctg	9720
gtgtggccac	ccggctggtt	ctgggtcttg	tagtgagtgc	aaaccccccc	tagggaaagg	9780
cacaagccct	ctagatgagg	tgttagaaca	agtcctctat	aagcctccac	ggactgtaat	9840
catgcatgtg	gagcagggtc	tcacccctct	tgaccagggc	agataccaga	ctcgccgcgg	9900
attagtctcc	gttaggcgtg	gcacagaggg	aaatgaagtt	gacctaccag	acgggtgatta	9960
tgctagcacc	gccctactcc	ccacttgtaa	agagatcaac	atggctcgctg	tcgctcttaa	10020
tgtgttgccg	agcagggtca	tcacgggtcc	gcccgggtgt	gggaaaacat	actggctcct	10080
tcagcagggtc	caggatgggtg	atgtcattta	cacaccgact	caccagacca	tgtctgacat	10140
gattagggct	ttggggacgt	gccgggtcaa	cgtcccagca	ggtacaacgc	tgcaattccc	10200
tgccccctcc	cgtaccggcc	cgtgggttcg	catcctagcc	ggcggttggt	gtcctggtaa	10260
gaattccttc	ttgatgaag	cagcgtattg	taatcacctt	gatgtcttga	ggctccttag	10320
caaaaccacc	ctcacctgtc	tgggagactt	caaacaactc	caccagtggt	gttttgattc	10380
tcattgctat	gtttttgaca	tcattgcctc	gaccagttg	aagaccatct	ggagattcgg	10440
acagaacatc	tgtgatgcca	tccaaccaga	ttacagggac	aaacttgtgt	ccatgggtcaa	10500
cacaaccctg	gtaacctacg	tggaaaaacc	tgtcaagtat	gggcaagtcc	tcacccctta	10560
ccacagggac	cgagaggacg	gcgccatcac	aattgactcc	agtcaaggcg	ccacatttga	10620
tgtggtcaca	ctgcatttgc	ccactaaaga	ttcactcaac	aggcaaagag	cccttggtgc	10680
tatcaccagg	gcaagacatg	ctatctttgt	gtatgaccca	cacaggcaat	tgcagagcat	10740
gtttgatctt	cctgcgaagg	gcacaccctg	caacctcgca	gtgcaccgtg	atgagcagct	10800
gatcgtactg	gatagaaata	ataaagaatg	cacagttgct	caggctctag	gcaacggaga	10860
taaatttagg	gccaccgaca	agcgcgttgt	agattctctc	cgcgccattt	gtgctgatct	10920

-continued

ggaagggctcg	agctctccgc	tccccaaagg	cgcacacaac	ttgggatttt	atttctcacc	10980
tgattttgaca	cagtttgcta	aactcccgg	agaccttgca	ccccactggc	ccgtgggtgac	11040
aaccacagaac	aatgaaaagt	ggccggatcg	gctgggttgc	agccttcgcc	ctgtccataa	11100
gtatagccgt	gcgtgcattg	gtgccggcta	tatgggtggc	ccctcggtgt	ttctaggcac	11160
ccctgggggtc	gtgtcatact	acctcacaaa	atttgtcaag	ggcgaggctc	aagtgttcc	11220
ggagacagtc	ttcagcaccc	gccgaattga	ggtggattgc	cgaggagtac	ttgatgacag	11280
ggagcgagaa	gttgctgagt	ccctcccaca	tgcttccatt	ggcgacgtca	aaggcaccac	11340
cgttggggga	tgtcatcatg	tcacctccaa	ataccttcgc	cgcttccttc	ccaaggaatc	11400
agtcgcggta	gtcgggggtt	cgagccccgc	gaaagccgca	aaagcagtgt	gcacattgac	11460
ggatgtgtac	ctcccagacc	ttgaggccta	cctccacca	gagactcagt	ccaagtgtctg	11520
gaaagtatatg	ttggacttca	aggaagtctg	actgatggtc	tggaaagaca	agacggccta	11580
ttccaactt	gaaggccgct	atttcacctg	gtatcagctt	gcaagctacg	cctcgtacat	11640
ccgtgttct	gtcaactcca	cgggtgtatc	ggacccctgc	atgggccttg	ccctttgcaa	11700
cagaagagtt	gtcgggtcca	ccattgggg	agtgacctc	gcagtcaccc	cttatgatta	11760
cggtgctaaa	atcatcttgt	ctagcgctta	ccatggtgaa	atgcctcctg	gatacaagat	11820
tctggcgtgc	gcgaggttct	cgctcgacga	cccagtcagg	tacaaacaca	cctgggggtt	11880
tgaatcggat	acagcgtatc	tgtatgagtt	caccggaaac	ggtgaggact	gggaggatta	11940
caatgatgcg	tttcgtgctg	gccagaaagg	gaaaatttat	aaggccactg	ctaccagcat	12000
gaagttttat	tttcccccg	gccccgtcat	tgaaccaact	ttaggcctga	attgaaatga	12060
aatgggggtct	atacaaagcc	tcttcgacaa	aattggccag	ctttttgtgg	atgttttcac	12120
ggaatttttg	gtgtccattg	ttgatatcat	catatttttg	gccattttgt	ttggcttcac	12180
catcgccgg	tggctgggtg	tcttttgcat	cagattgggt	tgctccgcgg	tattccgtgc	12240
gcgccctgcc	attcaccttg	agcaattaca	gaagatccta	tgaggccctt	ctttctcagt	12300
gccgggtgga	cattcccacc	tggggggtaa	aacaccctt	ggggatgttt	tggcaccata	12360
aggtgtcaac	cctgattgat	gaaatgggtg	cgcgtcgaat	gtaccgcac	atggaaaaag	12420
cagggcaagc	tgcttgaaa	cagggtggta	gcgaggctac	gctgtctcgc	attagtagtt	12480
tggatgtggt	ggctcatttt	caacatcttg	ccgccattga	agccgagacc	tgtaaatatt	12540
tggcttctcg	actgcccatt	ctacacaacc	tgcgcatgac	agggtcacaa	gtaaccatag	12600
tgtataatag	cactttaaat	cagggtgtttg	ctatttttcc	aaccctgggt	tcccggccaa	12660
agcttcatga	ttttcagcaa	tggctaatag	ctgtacattc	ctccatattt	tcctctgttg	12720
cagcttcttg	tactcttttt	gttgtgctgt	ggttgccggg	tccaatgcta	cgtactgttt	12780
ttggtttccg	ctggttaggg	gcaatttttc	tttcgaactc	atgggtgaatt	acacgggtgtg	12840
tccaccttgc	ctcaccgcac	aagcagccgc	tgaggctcct	gaaccgggta	ggtctctttg	12900
gtgcaggata	gggcatgacc	gatgtgggga	ggacgatcac	gacgaactgg	ggttcattgg	12960
tccgcctggc	ctctccagcg	aaagccactt	gaccagtgtt	tacgcctggg	tggcgcttct	13020
gtccttcagc	tacacggccc	agttccatcc	cgagatattt	gggataggga	acgtgagtga	13080
agtttatggt	gacatcaagc	accaattcat	ctgcgcggtt	catgacgggc	agaacaccac	13140
cttgccctgc	catgacaata	tttcagccgt	atttcagacc	tactatcaac	atcagggtcg	13200
cggcggaat	tggtttcacc	tagaatggct	gcgtcccttc	tttctctctt	ggttggtttt	13260

-continued

aaatgtttcg	tggtttctca	ggcgttcgcc	tgcaagccat	gtttcagttc	gagtcctttca	13320
gacatcaaaa	ccaacactac	cgcagcatca	ggctttgttg	tcctccagga	catcagctgc	13380
cttaggcattg	gcgactcgtc	ctttccgacg	attcgcaaaa	gctctcaatg	ccgcacggcg	13440
atagggacac	ctgtgtatat	caccatcaca	gccaatgtga	cagatgagaa	ttacttacat	13500
tctttctgatc	tcctcatgct	ttcttcttgc	cttttctatg	cttctgagat	gagtgaaaag	13560
ggattcaagg	tggtatttgg	caatgtgtca	ggcatcgtgg	ctgtgtgtgt	caactttacc	13620
agctacgtcc	aacatgtcaa	agagtttacc	caacgctcct	tggtggtcga	tcatgtgcgg	13680
ctgcttcatt	tcatgacacc	tgagaccatg	aggtgggcaa	ccgttttagc	ctgtcttttt	13740
gccatcctac	tggaatttg	aatgttcaag	tatgttgggg	aaatgcttga	ccgcgggctg	13800
ttgctcgcga	ttgctttctt	tgtgggtgat	cgtgccgttc	tggtttgctg	tgctcggcaa	13860
cgccaacagc	agcagcagct	ctcattttca	gttgatttat	aacttgacgc	tatgtgagct	13920
gaatggcaca	gattggctgg	cagaaaaatt	tgattgggca	gtggagactt	ttgtcatctt	13980
tcccggtgttg	actcacattg	tttcctatgg	tgcaactcacc	accagccatt	tccttgacac	14040
agttgggtctg	gttactgtgt	ccaccgccgg	gttttatcac	gggcgggatg	tcttgagtag	14100
catctacgcg	gtctgtgctc	tggtgcgtt	gatttgcttc	gttattaggc	ttgcgaagaa	14160
ctgcatgtcc	tggcgctact	cttgtagcag	atataccaac	ttccttcttg	acactaaggg	14220
cagactctat	cgttggcggt	cgcgcgttat	catagaaaaa	gggggtaagg	ttgaggtcga	14280
aggtcatctg	atcgacctca	aaagagttgt	gcttgatggg	tccgtggcaa	cccctttaac	14340
cagagtttca	gcggaacaat	ggggtcgtct	ctagacgact	tttgccatga	tagcacggct	14400
ccacaaaagg	tgcttttggc	gttttccatt	acctacacgc	cagtaatgat	atatgctcta	14460
aaggtaagtc	gcggccgact	gctagggctt	ctgcaccttt	tgatctttct	gaattgtgct	14520
tttaccttcg	ggtagatgac	attcgcgcac	tttcagagca	caaatagggt	cgcgctcgct	14580
atgggagcag	tagttgcact	tctttggggg	gtgtactcag	ccatagaaac	ctggaaattc	14640
atcacctcca	gatgccgttt	gtgcttgcta	ggccgcaagt	acattctggc	ccctgcccac	14700
cacgtcgaaa	gtgccgcggg	ctttcatccg	attgcggcaa	atgataacca	cgcatttgtc	14760
gtccggcgtc	ccggctccat	tacggttaac	ggcacattgg	tgcccggggt	gaaaagcctc	14820
gtgttggggtg	gcagaaaagc	tgttaaacag	ggagtggtaa	accttgctca	atatgccaaa	14880
taacaacggc	aagcagcaaa	agaaaaagaa	ggggaatggc	cagccagtca	accagctgtg	14940
ccagatgctg	ggtaaaatca	tcgcccagca	aaaccagtc	agaggcaagg	gaccgggctc	15000
taagaagaaa	tccccggaga	agccccattt	tcctctagcg	accgaagatg	acgtcaggca	15060
tcacttcacc	cctgggtgagc	ggcaattgtg	tctgtcgctg	atccagactg	cctttaacca	15120
gggcgctgga	acttgtagcc	tgtcagattc	agggaggata	agttacactg	tggagttag	15180
tttgccgacg	catcatactg	tgcgctgat	ccgcgtcaca	gcataccct	cagcatgatg	15240
ggctggcatt	ctttaggcac	ctcagtgtca	gaattggaag	aatgtgtggt	ggatggcact	15300
gattgacatt	gtgcctctaa	gtcacctatt	caattagggc	gaccgtgtgg	gggtaaaatt	15360
taattggcga	gaaccatgcg	gccgcaatta	aaaaaaaaa	aaaaaaaaa	aaaaaaaaa	15420
aaaaaaaaa	aaaaaaaaa	aaaa				15444

<210> SEQ ID NO 3
<211> LENGTH: 15450
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:

<223> OTHER INFORMATION: P129-PK-FL passage 24 complete genome (15,450 nt) full length vaccine

<400> SEQUENCE: 3

```

atgacgtata ggtggttgct ctatgccacg gcattttgtat tgtcaggagc tgtgaccatt    60
ggcacagccc aaaacttgct gcacggaaaa cgcccttctg tgacagcctt cttcagggga    120
gcttaggggt ctgtccctag caccttgctt ctggagttgc actgctttac ggtctctcca    180
cccctttaac catgtctggg ataacttgatc ggtgcacgtg ccccccaat gccaggggtgt    240
ttatggcgga gggccaagtc tactgcacac gatgtctcag tgcacggctc ctccttcctc    300
tgaatctcca agttcctgag cttgggggtgc tgggcctatt ttataggccc gaagagccac    360
tccggtggac gttgccacgt gcattcccca ctgtcgagtg cccccctgcc ggggcctgct    420
ggctttctgc gatctttcca attgcacgaa tgaccagtgg aaacctgaac tttcaacaaa    480
gaatggtgcg ggttgacagt gagatttaca gagccggcca actcaccctc gcagttttga    540
aggctctaca agtttatgaa cgggggttgc gctggtaccc cattgtcggc cctgtccctg    600
gagtggccgt ttacgccaac tccctacatg tgagtgacaa acctttcccg ggagcaactc    660
atgtgttaac caacctaccg ctcccgagcaggcccaagcc tgaagacttt tgcccttttg    720
agtgtgctat ggctaacgtc tatgacattg gccataacgc cgtcatgtat gtggccagag    780
ggaaagtctc ctgggcccct cgtggcgggg atgaagtga atttgaaacc gtccccgaag    840
agttgaagtt gattgcgaac cgactccaca tctccttccc gccccaccac gcagtggaca    900
tgtctgagtt tgccttcata gcccctggga gtggtgtctc cttgcgggtc gagcaccaac    960
acggctgect tcccgtgat actgtccctg atgggaaactg ctgggtgttac ttgtttgact   1020
tgctcccacc ggaagttcag aataaagaaa ttcgccgtgc taaccaattt ggctatcaaa   1080
ccaagcatgg tgtccatggc aagtacctac agcggaggct gcaagttaat ggtctccgag   1140
cagtgaactga tacagatgga cctattgtcg tacagtactt ctctgttagg gagagttgga   1200
tccgccactt cagactggcg gaagaacctc gcctccctgg gtttgaagac ctctcagaa   1260
taagggtaga gcctaatacg tcgccaatgg gtggcaaggg tgaaaaaatc ttccggtttg   1320
gcagtcacaa gtggtacggt gctggaaaga gagcaaggag agcacgctct ggtgcgactg   1380
ccacggctgc tcaccgctt ttgcccgtc gcgaagccca gcaggccaag aagctcgagg   1440
ttgccagcgc caacagggct gagcatctca agtactatc cccgcctgcc gacgggaact   1500
gtggtttgga ctgcatttcc gccattacca accggatggg gaattccaaa tttgaaacca   1560
ctcttcccga gagagtgaga ccttcagatg actgggctac tgacgaggat cttgtgaata   1620
ccatccaaat cctcaggctc cccgcggcct tggacaggaa cggtgcttgt gctggcgcca   1680
agtacgtgct caagctggaa ggtgagcact ggaccgtctc tgtgaccctt gggatgacct   1740
cttctttgct ccccctgaa tgtgttcagg gttgttgtga gcataagagc ggtcttggtt   1800
tcccagacgt ggtcgaagtt tccggatttg accctgcttg tcttgaccga cttgctgaga   1860
taatgcactt gcctagcagt gtcaccccag ctgctctggc cgagatgtcc gacgacttca   1920
atcgtctggc ttccccggcc gccactgtgt ggactgtttc gcaattcttt gcccgccaca   1980
gaggaggaga gcatcctgac cagggtgtgct tagggaaaat tatcaacctt tgtcaggtga   2040
ttgaggaatg ctgctgttcc cggaacaaag ccaaccgggc taccceggaa gaggttgagg   2100
caaaagttga ccagtacctc cgtggtgcag caagccttgg agaatgcttg gccaaagcttg   2160
agagggctcg cccgccgagc gcgacggaca cctcctttga ttggaatgtt gtgcttctg    2220

```

-continued

gggttgagac	ggcgaatcag	acaaccaaac	agctccatgt	caaccagtgc	cgcgctctgg	2280
ttcctgtcgt	gactcaagag	cctttggaca	gagactcggg	cctctgacc	gccttctcgc	2340
tgtccaattg	ctactaccct	gcacaagggtg	acgagggtccg	tcaccgtgag	aggctaaact	2400
ccttgctctc	taagttggag	ggggttgttc	gtgaggaata	tgggctcacg	ccaactggac	2460
ctggcccgcg	acccgcactg	cgaacgggc	tcgacgagct	taaagaccag	atggaggagg	2520
atctgctgaa	attagtcaac	gcccaggcaa	cttcagaaat	gatggcctgg	gcagccgagc	2580
aggttgatct	aaaagcttgg	gtcaaaaatt	accacgggtg	gacaccgcca	ccccctccac	2640
caagagttca	gcctcgaaaa	acgaagtctg	tcaagagctt	gctagagaac	aagcctgtcc	2700
ctgctccgcg	caggaaggtc	agatctgatt	gtggcagccc	gattttgatg	ggcgacaatg	2760
ttcctaacgg	ttgggaagat	tgcactgttg	gtggtccct	tgatctttcg	gcaccatccg	2820
agccgatgac	acctctgagt	gagcctgtac	ttatttccag	gccagtgaca	tctttgagtg	2880
tgcggcccc	agttcctgca	cgcgtagag	ctgtgtcccg	accgatgacg	ccctcgagtg	2940
agccaatttt	tgtgtctgca	ctgcgacaca	aatttcagca	ggtggaaaaa	gcaaactctgg	3000
cggcagcagc	gccgatgtgc	caggacgaac	ccttagattt	gtctgcatcc	tcacagactg	3060
aatatgaggg	ttcccccta	acaccaccgc	agaacgtggg	cattctggag	gtaagggggc	3120
aagaagctga	ggaagtctg	agtgaatct	cggatattct	gaatgatacc	aacctgcac	3180
ctgtgtcatc	aagcagctcc	ctgtcaagtg	ttaagatcac	acgccccaaa	tactcagctc	3240
aagccattat	cgactcgggc	gggccctgca	gtgggcacct	ccaaaggga	aaagaagcat	3300
gcctccgcat	catgcgtgag	gcttgtgatg	cggccaagct	tagtgaccct	gccacgcagg	3360
aatggctttc	tcgcatgtgg	gataggggtg	acatgctgac	ttggcgcaac	acgtctgctt	3420
accaggcggt	tcgcacctta	gatggcaggt	ttgggtttct	cccaaagatg	atactcgaga	3480
cgcgcgcgc	ctaccctgtg	gggtttgtga	tgttgctca	cacctctgca	ccttccgtga	3540
gtgcagagag	cgaccttacc	attggttcag	tcgccactga	agatattcca	cgcacctcgc	3600
ggaaaataga	aaataccggt	gagatgatca	accagggaac	cttggcatcc	tctgaggaag	3660
aaccggtata	caaccaacct	gccaaagact	cccgatatc	gtcgcggggg	tctgacgaga	3720
gcacagcagc	tccgtccgcg	ggtacagggtg	gcgcgggctt	atttactgat	ttgccacctt	3780
cagacggcgt	agatgcggac	ggtggggggc	cgttgacagc	ggtaagaaag	aaagctgaaa	3840
ggctcttcga	ccaattgagc	cgtcagggtt	ttaacctcgt	ctcccatctc	cctgttttct	3900
tctcacacct	cttcaaactc	gacagtgggt	attctccggg	tgattggggg	tttgcagctt	3960
ttactctatt	ttgcctcttt	ttgtgttaca	gctacccatt	cttcggtttc	gttccccctc	4020
tgggtgtatt	ttctgggtct	tctcggcggtg	tcgcgatggg	ggtttttggc	tgttggtgg	4080
cttttgctgt	tggcctgttc	aagcctgtgt	ccgacccagt	cggcactgct	tgtgagtttg	4140
actcgccaga	gtgcaggaa	gtccttcatt	cttttgagct	tctcaaacct	tgggaccttg	4200
ttcgcagcct	tgttggtggc	cccgtcggtc	tcggtcttgc	cattcttggc	aggttactgg	4260
gcggggcacg	ctacatctgg	cattttttgc	ttaggcttgg	cattgttgca	gattgtatct	4320
tggctggagc	ttatgtgctt	tctcaaggta	ggtgtaaaaa	gtgctgggga	tcttgataaa	4380
gaactgctcc	taatgaaatc	gccttcaacg	tgttcccttt	tacacgtgcg	accaggctcg	4440
cactcatcga	cctgtgcgat	cggttttgtg	cgccaacagg	catggacccc	attttcctcg	4500
ccactgggtg	gcgtgggtgc	tggaccggcc	gaagtcccat	tgagcaaccc	tctgaaaaac	4560

-continued

ccatcgcggtt	cgcccagttg	gatgaaaaga	ggattacggc	tagaactgtg	gtcgctcagc	4620
cttatgatcc	taatcaagcc	gtgaagtgct	tgcgggtggt	acaggcgggg	ggggcgatgg	4680
tggccgaggg	agtcccaaaa	gtggccaaag	tttctgctat	tccattccga	gccccTTTTT	4740
ttcccaccgg	agtgaaagtt	gatcccagat	gcaggatcgt	ggttgacccc	gataactTTTa	4800
ctacagccct	cgggtctggt	tactctacca	caaacctcgt	ccttggtgtg	ggggactttg	4860
cccagctgaa	tggactaaa	atcaggcaaa	tttccaagcc	ttcgggagga	ggcccacacc	4920
tcattgtgc	cctgcatgtt	gcctgctcga	tggcgttgca	catgcttgc	ggggtttatg	4980
taacttcagt	ggggtcttgc	ggtgccggca	ccaacgatcc	atggtgcact	aatccgtttg	5040
ccgttcctgg	ctacggacca	ggctctctct	gcacgtccag	attgtgcac	tcccaacatg	5100
gccttaccct	gcccttgaca	gcacttgtgg	cgggattcgg	tcttcaggaa	atcgcttgg	5160
tcgttttgat	tttcgtttcc	atcggaggca	tggctcatag	gttgagttgt	aaggctgata	5220
tgtgtgcat	tttacttgca	atcgccagct	atgtttgggt	acccttacc	tggttgcttt	5280
gtgtgtttcc	ttgttggttg	cgtcgttct	ctttgcaccc	ccttaccatc	ctatggttgg	5340
tgtttttctt	gatttctgta	aatatgcctt	cgggaatctt	ggcgtgggtg	ttattggttt	5400
ctctttggct	tttgggacgt	tatactaaca	ttgtcgtct	tgtcaccccc	tatgatattc	5460
atcattacac	cagtggcccc	cgcgggtgtg	ccgccttggc	taccgcacca	gatggaacct	5520
acttggtgc	cgtccgccgc	gctgcgttga	ctggtcgcac	catgctgttc	accccgcttc	5580
agcttgggtc	ccttcttgag	ggcgctttca	gaactcgaaa	gccctcactg	aacaccgtca	5640
atgtggttgg	gtcctccatg	ggctctgggt	gagtgttcac	catcgacggg	aaaattaggt	5700
gcgtgactgc	cgcacatgtc	cttacgagta	attcggttag	ggtttcggga	gtcggcttca	5760
atcaaagtct	tgactttgat	gtgaaagggg	acttcgccat	agctgattgc	cgaattggc	5820
aaggagctgc	tcccaagacc	caattctgcg	aggacggatg	gactggccgt	gcctattggc	5880
tgacatcctc	tggcgtcgaa	cccgggtgta	ttgggaatgg	attcgcttc	tgettccaccg	5940
cgtgcggcga	ttccgggtcc	ccagtgatca	ccgaagctgg	tgagattgtc	ggcggtcaca	6000
caggatcaaa	taaacaagga	ggtggcatcg	tcacgcgccc	ttcaggccag	ttttgtaacg	6060
tggcacccat	caagctgagc	gaattaagtg	aattctttgc	tggaccaag	gtcccgcctc	6120
gtgatgtgaa	ggttggcagc	cacataatta	aagacacgtg	cgaagtacct	tcagatcttt	6180
gcgccttgct	tgctgccaaa	cctgaactgg	agggaggcct	ctccaccgtc	caacttctgt	6240
gtgtgttttt	cctactgtgg	agaatgatgg	gacatgcctg	gacgcccttg	gttgcgtgtg	6300
ggtttttcat	tctgaatgag	gttctcccag	ctgtcctgg	tcggagtgtt	ttctcctttg	6360
ggatgtttgt	gctatcttgg	ctcacaccat	ggtctgcgca	agttctgatg	atcaggcttc	6420
taacagcagc	tcttaacagg	aacagatgg	cacttgccct	ttacagcctt	ggtgcgggtga	6480
ccggttttgt	cgcagatctt	gcggtaactc	aagggcaccc	gttgcaggca	gtaatgaatt	6540
tgagcaccta	tgccttcctg	cctcggtatga	tggttgtgac	ctcaccagtc	ccagtgattg	6600
cgtgtggtgt	tgtgcaccta	cttgccatca	ttttgtactt	gttcaagtac	cgcggcctgc	6660
acaatgttct	tgttggtgat	ggagcgtttt	ctgcagcttt	cttcttgcca	tactttgccg	6720
agggaaagtt	gagggaagg	gtgtcgcaat	cctgcggaat	gaatcatgag	tcattgactg	6780
gtgccctcgc	tatgagactc	aatgacgagg	acttggaact	ccttacgaaa	tggactgatt	6840
ttaagtgtt	tgtttctgcg	tccaacatga	ggaatgcagc	aggccaattc	atcagggtg	6900
cctatgcaaa	agcacttaga	attgaacttg	cccagttgg	gcaggttgat	aaggttcgag	6960

-continued

gtactttggc	caagcttgag	gcttttgctg	ataccgtggc	accccaactc	tcgcccgggtg	7020
acattgttgt	tgctcttggc	catacgcttg	ttggcagcat	cttcgaccta	aaggttggtg	7080
gtaccaagca	tactctccaa	gtcattgaga	ccagagtcct	tgccgggtcc	aaaatgaccg	7140
tggcgcgcgt	cgttgacca	acccccacgc	ccccaccgc	acccgtgccc	atccccctcc	7200
caccgaaagt	tctagagaat	ggtcccaacg	cctgggggga	tggggaccgt	ttgaataaga	7260
agaagaggcg	taggatggaa	accgtcggca	tctttgtcat	gggtgggaag	aagtaccaga	7320
aattttggga	caagaattcc	ggtgatgtgt	tttacgagga	ggtccatgac	aacacagatg	7380
cgtgggagtg	cctcagagtt	ggtgaccctg	ccgactttaa	ccctgagaag	ggaactctgt	7440
gtgggcatac	tactattgaa	gataaggatt	acaaagtcta	cgctcccca	tctggcaaga	7500
agttcctggg	ccccgtcaac	ccagagagcg	gaagagccca	atgggaagct	gcaaagcttt	7560
ccgtggagca	ggcccttggc	atgatgaatg	tcgacgggtg	actgacggcc	aaagaagtgg	7620
agaaactgaa	aagaataatt	gacaaacttc	agggcctgac	taaggagcag	tgtttaaact	7680
gctagccgcc	agcggcttga	cccgtgtgg	tcgcggcggc	ttggttggtta	ctgagacagc	7740
ggtaaaaata	gtcaaatttc	acaaccggac	tttcacccta	gggcctgtga	atttaaaagt	7800
ggccagtgag	gttgagctga	aagacgcggg	cgagcacaac	caacaccggg	ttgcaagacc	7860
ggttgacggg	ggtgttgtgc	tctgcgttc	cgcagtctct	tcgcttatag	atgtcctgat	7920
ctccgggtgct	gacgcctctc	ctaagttact	cgctcgtcac	gggccgggga	acactgggat	7980
cgatggcacg	ctttgggact	ttgaggccga	ggccaccaa	gaggaaattg	cgctcagtgc	8040
gcaaataata	caggcttgtg	acattaggcg	cggtgacgca	cctgaaattg	gtctccctta	8100
caagctgtac	cctgttaggg	gcaaccctga	gcgggtaaaa	ggagttttac	agaatacaag	8160
gtttggagac	ataccttaca	aaacccccag	tgacactggg	agcccagtgc	acgcggctgc	8220
ctgcctcacg	ccaatgcca	ctccgggtgac	tgatgggcgc	tccgtcttgg	ctactaccat	8280
gccctccggg	tttgaattgt	atgtaccgac	cattccagcg	tctgtccttg	attatcttga	8340
ctctaggcct	gactgcccc	aacagttgac	agagcacggc	tgtgaggatg	ccgcattgag	8400
agacctctcc	aagtatgact	tgtccacca	aggctttggt	ttacctgggg	ttcttcgcct	8460
tgtgcgtaag	tacctgtttg	cccacgtggg	taagtgcgcc	ccggttcac	ggccttcac	8520
ttacctgcc	aagaattcta	tggctggaat	aaatgggaac	aggtttcaa	ccaaggacat	8580
tcagagcgtc	cccgaaatcg	acgttctgtg	cgcacaggcc	gtgcgagaaa	actggcaaac	8640
tgttaccct	tgtaccctca	agaaacagta	ttgtgggaag	aagaagacta	ggacaatact	8700
cggcaccaat	aatttcattg	cgttggccca	ccgggcagcg	ttgagtgggtg	tcaccagggtg	8760
cttcatgaaa	aaggcgttta	actcgcccat	cgccctcggg	aaaaacaaat	ttaaggagct	8820
acagactccg	atcttaggca	ggtgccttga	agctgatctt	gcacctctgtg	atcgatccac	8880
acctgcaatt	gtccgctggg	ttgccgcaa	ccttctttat	gaacttgcct	gtgctgaaga	8940
gcacctaccg	tcgtacgtgc	tgaactgctg	ccatgaccta	ttggtcacgc	agtcgggcgc	9000
agtgactaag	aggggtggcc	tgtcgtctgg	cgaccgcatc	acttctgtgt	ctaaccacat	9060
ttacagcttg	gtgatatatg	cacagcacat	ggtgcttagt	tactttaaaa	gtggtcacc	9120
tcatggcctt	ctgttcctac	aagaccagct	gaagtctgag	gacatgctca	aagtccaacc	9180
cctgatcgtc	tattcggacg	acctcgtgct	gtatgccgaa	tctcccacca	tgccgaacta	9240
ccactggtgg	gtcgaacatc	tgaatttgat	gctgggtttt	cagacggacc	caaagaagac	9300

-continued

agccataacg	gactcgccat	catttctagg	ctgtaggata	ataaatggac	gccagctagt	9360
ccccaaccgt	gacaggatcc	tgcgggccct	cgcttaccat	atgaaggcaa	gcaatgtttc	9420
tgaatactac	gccgcggcgg	ctgcaatact	catggacagc	tgtgcttggt	tagagtatga	9480
tcctgaatgg	tttgaagagc	ttgtgggttg	gatagcgcag	tgcgcccgc	aggacggcta	9540
cagctttccc	ggcccggcgt	tcttcttgtc	catgtgggaa	aaactcagat	ccaatcatga	9600
ggggaagaag	tccagaatgt	gcgggtattg	cggggccccg	gctccgtacg	ccactgcctg	9660
tggcctcgac	gtctgtat	accacacca	cttccaccag	cattgtccag	tcacaatctg	9720
gtgtggccac	ccggctggtt	ctggttcttg	tagtgagtgc	aaaccccccc	tagggaaagg	9780
cacaagccct	ctagatgagg	tgtagaaca	agtcccgtat	aagcctccac	ggactgtaat	9840
catgcatgtg	gagcagggtc	tcacctctct	tgaccaggc	agataccaga	ctcgccgcgg	9900
attagtctcc	gttaggcgtg	gcacagagg	aatgaagtt	gacctaccag	acggtgatta	9960
tgctagcacc	gccctactcc	ccacttgtaa	agagatcaac	atggtcgctg	tcgctctaa	10020
tgtgttgccg	agcaggttca	tcacgggtcc	gcccgggtgt	gggaaaacat	actggctcct	10080
tcagcaggtc	caggatggtg	atgtcattta	cacaccgact	caccagacca	tgctcgacat	10140
gattagggct	ttggggacgt	gccggttcaa	cgtcccagca	ggtacaacgc	tgcaattccc	10200
tgccccctcc	cgtaccggcc	cgtgggttcg	catcctagcc	ggcggttggt	gtcctggtaa	10260
gaattccttc	ttggatgaag	cagcgtattg	taatcacctt	gatgtcttga	ggctccttag	10320
caaaaccacc	ctcacctgtc	tgggagactt	caaacaactc	caccagtggt	gttttgattc	10380
tcattgctat	gtttttgaca	tcatgcctca	gaccagttg	aagaccatct	ggagattcgg	10440
acagaacatc	tgtgatgcca	tccaaccaga	ttacagggac	aaacttgtgt	ccatgggtcaa	10500
cacaaccctg	gtaacctacg	tggaaaaacc	tgtaagtat	gggcaagtcc	tcaccttcta	10560
ccacaggggac	cgagaggacg	gcgccatcac	aattgactcc	agtcaaggcg	ccacatttga	10620
tgtggtcaca	ctgcatttgc	ccactaaaga	ttcactcaac	aggcaaagag	cccttggtgc	10680
tatcaccagg	gcaagacatg	ctatctttgt	gtatgaccca	cacaggcaat	tgacagagcat	10740
gtttgatctt	cctgcgaagg	gcacaccctg	caacctcgca	gtgcaccgtg	atgagcagct	10800
gacgtactg	gatagaaata	ataaagaatg	cacagttgct	caggctctag	gcaacggaga	10860
taaatttagg	gccaccgaca	agcgcgttgt	agattctctc	cgcgccattt	gtgctgatct	10920
ggaagggctg	agctctccgc	tccccagggt	cgcacacaac	ttgggatttt	atttctcacc	10980
tgatttgaca	cagtttgcta	aactcccggg	agaccttgca	ccccactggc	ccgtgggtgac	11040
aacccagAAC	aatgaaaagt	ggccggatcg	gctgggtgcc	agccttcgcc	ctgtccataa	11100
gtatagccgt	gcgtgcattg	gtgccggcta	tatggtgggc	ccctcggtgt	ttctaggcac	11160
ccctgggggtc	gtgtcatact	acctcacaaa	atttgtcaag	ggcgaggctc	aagtgttcc	11220
ggagacagtc	ttcagcaccg	gccgaattga	ggtggattgc	cgggagtatc	ttgatgacag	11280
ggagcgagaa	gttgctgagt	ccctcccaca	tgccttcatt	ggcgacgtca	aaggcaccac	11340
cgttggggga	tgtcatcatg	tcacctccaa	ataccttcgg	cgttccttc	ccaaggaatc	11400
agtcgcggta	gtcgggggtt	cgagccccgg	gaaagccgca	aaagcagtgt	gcacattgac	11460
ggatgtgtac	ctcccagacc	ttgaggccta	cctccaccca	gagactcagt	ccaagtgtctg	11520
gaaagttagt	ttggacttca	aggaagtctg	actgatggtc	tggaaagaca	agacggccta	11580
tttccaactt	gaaggccgct	atttcacctg	gtatcagctt	gcaagctacg	cctcgtacat	11640
ccgtgttcct	gtcaactcca	cgggtgtatct	ggacccctgc	atgggccctg	ccctttgcaa	11700

-continued

cagaagagtt	gtcgggtcca	cccattgggg	agctgacctc	gcagtcaccc	cttatgatta	11760
cggtgctaaa	atcatcttgt	ctagcgctta	ccatggtgaa	atgcctcctg	gatacaagat	11820
tctggcgtgc	gcgagttct	cgctcgacga	cccagtcaag	tacaaacaca	cctgggggtt	11880
tgaatcggat	acagcgtatc	tgtatgagtt	caccggaaac	ggtgaggact	gggaggatta	11940
caatgatgcg	tttcgtgcgc	gccagaaagg	gaaaatttat	aaggccactg	ctaccagcat	12000
gaagttttat	tttcccccg	gccccgtcat	tgaaccaact	ttaggcctga	attgaaatga	12060
aatggggctc	atacaaagcc	tcttcgacaa	aattggccag	ctttttgtgg	atgctttcac	12120
ggaatttttg	gtgtccattg	ttgatatcat	catatttttg	gccattttgt	ttggcttcac	12180
catcgccggt	tggctgggtg	tcttttgcac	cagattgggt	tgctccgcgg	tattccgtgc	12240
gcgccctgcc	attcacctcg	agcaattaca	gaagatccta	tgaggccttt	ctttctcagt	12300
gccgggtgga	cattcccacc	tggggggtaa	aacacccttt	ggggatgttt	tggcaccata	12360
aggtgtcaac	cctgattgat	gaaatgggtg	cgcgtcgaat	gtaccgcatc	atggaaaaag	12420
cagggcaagc	tgcttggaag	caggtgggtg	gcgaggttac	gctgtctcgc	attagtagtt	12480
tggatgtggt	ggctcatttt	caacatcttg	ccgccattga	agccgagacc	tgtaaataat	12540
tggcttctcg	actgcccatg	ctacacaacc	tgcgcatgac	aggggtcaat	gtaaccatag	12600
tgtataatag	cactttaaat	caggtgtttg	ctattttttc	aacccttggt	tcccggccaa	12660
agcttcatga	ttttcagcaa	tggctaatag	ctgtacattc	ctccatattt	tcctctgttg	12720
cagcttcttg	tactcttttt	gttgtgctgt	ggttgccggg	tccaatgcta	cgtactgttt	12780
ttggtttccg	ctggttaggg	gcaatttttc	tttcgaactc	atgggtgaatt	acacgggtgtg	12840
tccaccttgc	ctcacccgac	aagcagccgc	tgaggctcct	gaaccgcgta	ggtctctttg	12900
gtgcaggata	gggcatgacc	gatgtgggga	ggacgatcac	gacgaactgg	ggttcatggg	12960
tccgcctggc	ctctccagcg	aaagccactt	gaccagtgtt	tacgcctggg	tggcggttct	13020
gtccttcagc	tacacggccc	agttccatcc	cgagatattt	gggataggga	acgtgagtga	13080
agtttatggt	gacatcaagc	accaattcat	ctgcgcggtt	catgacgggc	agaacaccac	13140
cttgccctcg	catgacaata	tttcagccgt	atttcagacc	tactatcaac	atcaggtcga	13200
cggcggcaat	tggtttcacc	tagaatggct	gcgtcccttc	tttctctctt	ggttggtttt	13260
aaatgtttcg	tggtttctca	ggcggttcgc	tgcaagccat	gtttcagttc	gagtctttca	13320
gacatcaaaa	ccaacactac	cgcagcatca	ggctttgttg	tcctccagga	catcagctgc	13380
cttaggcgat	gcgactcgtc	ctttccgacg	attcgcaaaa	gctctcaatg	ccgcacggcg	13440
atagggacac	ctgtgtatat	caccatcaca	gccaatgtga	cagatgagaa	ttacttacat	13500
tcttctgatc	tcctcatgct	ttcttcttgc	cttttctatg	cttctgagat	gagtgaaaag	13560
ggattcaagg	tggatatttg	caatgtgtca	ggcatcgtgg	ctgtgtgtgt	caactttacc	13620
agctacgtcc	aacatgtcaa	agagtttacc	caacgctcct	tgggtggtcga	tcattgtgcg	13680
ctgcttcatt	tcattgacac	tgagaccatg	aggtgggcaa	ccgttttagc	ctgtcttttt	13740
gccatcctac	tggcaatttg	aatgttcaag	tatgttgggg	aaatgcttga	ccgcgggctg	13800
ttgctcgoga	ttgctttctt	tgtgggtgat	cgtgccgttc	tggtttgctg	tgctcggcaa	13860
cgccaacagc	agcagcagct	ctcattttca	gttgatttat	aacttgacgc	tatgtgagct	13920
gaatggcaca	gattggctgg	cagaaaaatt	tgattgggca	gtggagactt	ttgtcatctt	13980
tcccgtgttg	actcacattg	tttctatgg	tgcactcacc	accagccatt	tccttgacac	14040

-continued

agttggtctg gttactgtgt ccaccgccgg gttttatcac gggcggtatg tcttgagtag	14100
catctacgcg gtctgtgctc tggtgcgtt gatttgcttc gttattaggc ttgcgaagaa	14160
ctgcatgtcc tggcgctact cttgtaccag atataccaac ttccttctgg acactaaggg	14220
cagactctat cgttggcggt cgcccgttat catagaaaaa gggggtaagg ttgaggtcga	14280
aggcatctg atcgacctca aaagagttgt gcttgatggg tccgtggcaa cccctttaac	14340
cagagtttca gcggaacaat ggggtcgtct ctagacgact tttgccatga tagcacggct	14400
ccacaaaagg tgcttttggc gttttccatt acctacacgc cagtaatgat atatgctcta	14460
aaggtaagtc gcggccgact gctagggctt ctgcaccttt tgatctttct gaattgtgct	14520
tttaccttcg ggtacatgac attcgcgcac tttcagagca caaatagggt cgcgctcgct	14580
atgggagcag tagttgcact tctttggggg gtgtactcag ccatagaaac ctggaaattc	14640
atcacctcca gatgccgttt gtgcttgcta ggccgcaagt acattctggc ccctgcccac	14700
cacgtcgaaa gtgccgcggg ctttcatccg attgcggcaa atgataacca cgcatttgct	14760
gtccggcgctc ccggctccat tacggttaac ggacattgg tgcccggggt gaaaagcctc	14820
gtgttggtg gcagaaaagc tgtaaacag ggagtggtaa accttgctca atatgccaaa	14880
taacaacggc aagcagcaaa agaaaaagaa ggggaatggc cagccagtca accagctgtg	14940
ccagatgctg ggtaaaatca tcgccagca aaaccagtc agaggcaagg gaccgggcaa	15000
gaaaagtaag aagaaaaacc cggagaagcc ccattttcct ctagcgaccg aagatgacgt	15060
caggcatcac ttcaccttg gtgagcggca attgtgtctg tcgtcgatcc agactgcctt	15120
taaccagggc gctggaactt gtacctgtc agattcaggg aggataagtt aactgtgga	15180
gtttagtttg ccgacgcac atactgtgc cctgatccgc gtcacagcat caccctcagc	15240
atgatgggct ggcattcttt aggcacctca gtgtcagaat tggaagaatg tgtggtggat	15300
ggcactgatt gacattgtgc ctctaagtca cctattcaat tagggcgacc gtgtgggggt	15360
aaaatttaat tggcgagaac catgcggccg caattaaaaa aaaaaaaaaa aaaaaaaaaa	15420
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa	15450

<210> SEQ ID NO 4
<211> LENGTH: 15444
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P129-PK-d43/44 passage 34 complete genome
(15,444 nt) 2 aa deleted vaccine

<400> SEQUENCE: 4

atgacgtata ggtgttggct ctatgccacg gcatttgtat tgtcaggagc tgtgaccatt	60
ggcacagccc aaaacttgct gcacggaaaa cgcccttctg tgacagcctt cttcagggga	120
gcttaggggt ctgtccctag caccttgctt ctggagttgc actgctttac ggtctctcca	180
cccctttaac catgtctggg atacttgatc ggtgcacgtg caccaccaat gccagggtgt	240
ttatggcgga gggccaagtc tactgcacac gatgtctcag tgcacggtct ctcttctctc	300
tgaatctcca agttcctgag cttgggggtgc tgggcctatt ttataggccc gaagagccac	360
tccggtggac gttgccacgt gcattcccca ctgtcgagtg ctcccctgcc ggggcctgct	420
ggctttctgc gatctttcca attgcacgaa tgaccagtgg aaacctgaac tttcaacaaa	480
gaatgggtgc ggttgacgct gagatttaca gagccggcca actcaccctc gcagttttga	540
aggctctaca agtttatgaa cgggggtgtc gctgggtacc cattgtcgga cctgtccctg	600

-continued

gagtggccgt	ttacgccaac	tccctacatg	tgagtgacaa	acctttcccg	ggagcaactc	660
atgtgttaac	caacctaccg	ctcccgcaga	ggcccaagcc	tgaagacttt	tgcccttttg	720
agtgtgctat	ggctaacgtc	tatgacattg	gccataacgc	cgtcatgtat	gtggccagaa	780
ggaaagtctc	ctgggcccct	cgtggcgggg	atgaagtgaa	atttgaaacc	gtccccgaag	840
agttgaagtt	gattgcgaac	cgactccaca	tctccttccc	gccccaccac	gcagtggaca	900
tgtctgagtt	tgccctcata	gcccctggga	gtggtgtctc	cttgccgggtc	gagcaccaac	960
acggctgect	tcccgtgat	actgtccctg	atgggaactg	ctgggtgttac	ttgtttgact	1020
tgctcccacc	ggaagttcag	aataaagaaa	ttcgccgtgc	taaccaattt	ggctatcaaa	1080
ccaagcatgg	tgtccatggc	aagtacctac	agcggaggct	gcaagttaat	ggtctccgag	1140
cagtgactga	tacagatgga	cctattgtcg	tacagtactt	ctctgttagg	gagagttgga	1200
tccgccactt	cagactggcg	gaagaacctc	gcctccctgg	gtttgaagac	ctcctcagaa	1260
taagggtaga	gcctaatacg	tcgccaatgg	gtggcaaggg	tgaaaaaatc	ttccggtttg	1320
gcagtacaaa	gtggtacggt	gctggaaaga	gagcaaggag	agcacgctct	ggtgcgactg	1380
ccacggtcgc	tcaccgcgct	ttgcccgctc	gcgaagccca	gcaggccaag	aagctcgagg	1440
ttgccagcgc	caacagggct	gagcatctca	agtactattc	cccgcctgcc	gacgggaact	1500
gtggttggca	ctgcatttcc	gccattacca	accggatggg	gaattccaaa	tttgaaacca	1560
ctcttcccga	gagagtgaga	ccttcagatg	actgggctac	tgacgaggat	cttgtgaata	1620
ccatccaaat	cctcaggctc	cccgcggcct	tggacaggaa	cgggtgcttg	gctggcgcca	1680
agtacgtgct	caagctggaa	ggtgagcact	ggaccgtctc	tgtgaccctc	gggatgaccc	1740
cttctttgct	cccccttgaa	tgtgttcagg	gttgttgtga	gcataagagc	ggtcttggtt	1800
ttccagacgt	ggtcgaagtt	tcgggatttg	accctgcctg	tcttgaccga	cttgctgaga	1860
taatgcactt	gcctagcagt	gtcatcccag	ctgctctggc	cgagatgtcc	gacgacttca	1920
atcgtctggc	ttccccggcc	gccactgtgt	ggactgtttc	gcaattcttt	gcccgccaca	1980
gaggaggaga	gcatacctgac	cagggtgtgct	tagggaaaat	tatcaacctt	tgtcagggtga	2040
ttgaggaatg	ctgctgttcc	cggaaacaaag	ccaaccgggc	taccccgga	gaggttgcgg	2100
caaaagttga	ccagtacctc	cgtgggtgcag	caagccttgg	agaatgcttg	gccaagcttg	2160
agagggctcg	cccgcggagc	gcgacggaca	cctcctttga	ttggaatgtt	gtgcttctctg	2220
gggttgagac	ggcgaatcag	acaaccaaac	agctccatgt	caaccagtgc	cgcgctctgg	2280
ttcctgtcgt	gactcaagag	cctttggaca	gagactcggg	ccctctgacc	gccttctcgc	2340
tgtccaattg	ctactaccct	gcacaaggtg	acgagggtccg	tcaccgtgag	aggctaaact	2400
ccttgctctc	taagttggag	ggggttgttc	gtgaggaata	tgggctcacg	ccaactggac	2460
ctggcccgcg	accgcactg	ccgaacgggc	tcgacgagct	taaagaccag	atggaggagg	2520
atctgctgaa	attagtcaac	gcccaggcaa	cttcagaaat	gatggccttg	gcagccgagc	2580
aggttgatct	aaaagcttgg	gtcaaaaatt	accacgggtg	gacaccgcca	ccccctccac	2640
caagagttca	gcctcgaaaa	acgaagtctg	tcaagagctt	gctagagaac	aagcctgtcc	2700
ctgctccgcg	caggaaggtc	agatctgatt	gtggcagccc	gattttgatg	ggcgacaatg	2760
ttcctaacgg	ttgggaagat	togactgttg	gtggtccctt	tgatctttcg	gcaccatccg	2820
agccgatgac	acctctgagt	gagcctgtac	ttattttccag	gccagtgaca	tctttgagtg	2880
tgccggcccc	agttcctgca	ccgcgtagag	ctgtgtcccc	accgatgacg	ccctcgagtg	2940
agccaatttt	tgtgtctgca	ctgcgacaca	aatttcagca	ggtggaaaaa	gcaaatctgg	3000

-continued

cggcagcagc	gccgatgtgc	caggacgaac	ccttagat	gtctgcatcc	tcacagactg	3060
aatatgagggc	ttcccccta	acaccaccgc	agaacgtggg	cattctggag	gtaagggggc	3120
aagaagctga	ggaagtctg	agtgaatct	cggatattct	gaatgatacc	aacctgcac	3180
ctgtgtcatc	aagcagctcc	ctgtcaagtg	ttaagatcac	acgccccaaa	tactcagctc	3240
aagccattat	cgactcgggc	gggccctgca	gtgggcacct	ccaaaggga	aaagaagcat	3300
gcctccgcac	catgcgtgag	gcttgatgag	cggccaagct	tagtgaccct	gccacgcagg	3360
aatggctttc	tcgcatgtgg	gataggggtg	acatgctgac	ttggcgcaac	acgtctgctt	3420
accaggcggt	tcgcacctta	gatggcaggt	ttgggtttct	cccaaagatg	atactcgaga	3480
cgcgcgcgc	ctaccctgtg	gggtttgtga	tgttgctca	cacctcgca	ccttcctgta	3540
gtgcagagag	cgaccttacc	attgggtcag	tcgccactga	agatattcca	cgcgtcctcg	3600
ggaaaataga	aaataccggt	gagatgatca	accagggaac	cttggcatcc	tctgaggaag	3660
aaccggtata	caaccaacct	gccccagact	cccggatata	gtcgcggggg	tctgacgaga	3720
gcacagcagc	cccgtccgcg	ggtacagggt	gcgcgggctt	atttactgat	ttgccacctt	3780
cagacggcgt	agatgcggac	ggtggggggc	cgttgacagc	ggtaagaaag	aaagctgaaa	3840
ggctcttcga	ccaattgagc	cgtcagggtt	ttaacctcgt	ctcccatctc	cctgttttct	3900
tctcacacct	cttcaaactc	gacagtgggt	attctccggg	tgattggggg	tttgcagctt	3960
ttactctatt	ttgcctcttt	ttgtgttaca	gtacccatt	cttcggtttc	gttccctct	4020
tgggtgtatt	ttctgggtct	tctcggcgtg	tgccatggg	ggtttttggc	tgttggtgg	4080
cttttctgt	tggcctgttc	aagcctgtgt	ccgaccagct	cggcactgct	tgtgagtttg	4140
actcgccaga	gtgcaggaac	gtccttcatt	cttttgagct	tctcaaacct	tgggaccctg	4200
ttcgcagcct	tgttggtggc	cccgtcggtc	tcggtcttgc	cattcttggc	aggttactgg	4260
gcggggcacg	ctacatctgg	cattttttgc	ttaggcttgg	cattgttgca	gattgtatct	4320
tggctggagc	ttatgtgctt	tctcaaggta	ggtgtaaaaa	gtgctgggga	tcttgataaa	4380
gaactgctcc	taatgaaatc	gccttcaacg	tgttcctttt	tacacgtgcg	accaggctgt	4440
cactcatcga	cctgtgcgat	cggttttgtg	cgccaacagg	catggacccc	attttcctcg	4500
ccactgggtg	gcgtgggtgc	tggaccggcc	gaagtcccat	tgagcaacct	tctgaaaaac	4560
ccatcgcggt	cggccagttg	gatgaaaaga	ggattacggc	tagaactgtg	gtcgtcagc	4620
cttatgatcc	taatcaagcc	gtgaagtgtc	tgccgggtgt	acaggcgggt	ggggcgatgg	4680
tggccgaggg	agtccccaaa	gtggccaaag	tttctgctat	tccattccga	gccccctttt	4740
ttcccaccgg	agtgaagtt	gatcccagat	gcaggatcgt	ggttgacccc	gatactttta	4800
ctacagccct	ccggtctggt	tactctacca	caaacctcgt	ccttggtgtg	ggggactttg	4860
cccagctgaa	tggactaaag	atcaggcaaa	tttccaagcc	ttcgggagga	ggcccacacc	4920
tcattgctgc	cctgcatgtt	gcctgctcga	tggcgttgca	catgcttgct	ggggtttatg	4980
taacttcagt	gggtcttgc	ggtgccggca	ccaacgatcc	atggtgcact	aatccgtttg	5040
ccgttcctgg	ctacggacca	ggctctctct	gcacgtccag	attgtgcata	tccaacatg	5100
gccttacct	gcccttgaca	gcacttggtg	cgggattcgg	tcttcaggaa	atcgcttggt	5160
tcgttttgat	tttcgtttcc	atcggaggca	tggctcatag	gttgagttgt	aaggctgata	5220
tgtgtgcat	tttacttgca	atcgccagct	atgtttgggt	accccttacc	tgggtgcttt	5280
gtgtgtttcc	ttgttggttg	cgtgggttct	ctttgcaccc	ccttaccatc	ctatgggttg	5340

-continued

tgtttttctt	gatttctgta	aatatgcctt	cggaatctt	ggcctgggtg	ttattggttt	5400
ctctttggct	tttgggacgt	tatactaaca	ttgtcgggtc	tgtcaccccc	tatgatattc	5460
atcattacac	cagtggcccc	cgcgggtgtg	ccgccttggc	taccgcacca	gatggaacct	5520
acttggetgc	cgtccgccgc	gctgcgttga	ctggtcgcac	catgctgttc	accccgcttc	5580
agcttgggtc	ccttcttgag	ggcgctttca	gaactcgaaa	gccctcactg	aacaccgtca	5640
atgtgggttg	gtcctccatg	ggctctgggt	gagtgttcac	catcgacggg	aaaattaggt	5700
gcgtgactgc	cgcacatgtc	cttacgagta	attcggttag	ggtttccgga	gtcggcttca	5760
atcaaagtct	tgactttgat	gtgaaagggg	acttcgccat	agctgattgc	ccgaattggc	5820
aaggagctgc	tcccaagacc	caattctgcg	aggacggatg	gactggccgt	gcctattggc	5880
tgacatcctc	tggcgtcgaa	cccgggtgta	ttgggaatgg	attcgccttc	tgcttcaccg	5940
cgtgcggcga	ttccgggtcc	ccagtgatca	ccgaagctgg	tgagattgtc	ggcggtcaca	6000
caggatcaaa	taaacaagga	ggtggcatcg	tcacgcgccc	ttcaggccag	ttttgtaacg	6060
tggcacccat	caagctgagc	gaattaagtg	aattctttgc	tggacccaag	gtcccgtctc	6120
gtgatgtgaa	ggttggcagc	cacataatta	aagacacgtg	cgaagtacct	tcagatcttt	6180
gcgccttgct	tgctgccaaa	cctgaactgg	agggaggcct	ctccaccgtc	caacttctgt	6240
gtgtgttttt	cctactgtgg	agaatgatgg	gacatgcctg	gacgccttgc	gttgcgtgtg	6300
ggtttttcat	tctgaatgag	gttctcccag	ctgtcctggg	tcggagtgtt	ttctcctttg	6360
ggatgtttgt	gctatcttgg	ctcacaccat	ggtctgcgca	agttctgatg	atcaggcttc	6420
taacagcagc	tcttaacagg	aacagatggg	cacttgcctt	ttacagcctt	ggtgcgggtga	6480
ccggttttgt	cgcagatctt	gcggtaactc	aagggcaccc	gttgcaggca	gtgatgaatt	6540
tgagcaccta	tgccttcctg	cctcggatga	tgggtgtgac	ctcaccagtc	ccagtgattg	6600
cgtgtgggtg	tgtgcaccta	cttgccatca	ttttgtactt	gttcaagtac	cgcggcctgc	6660
acaatgttct	tgttgggtgat	ggagcgtttt	ctgcagcttt	cttcttgcca	tactttgccg	6720
agggaaagtt	gaggggaagg	gtgtcgcaat	cctgcggaat	gaatcatgag	tcattaactg	6780
gtgccctcgc	tatgggactc	aatgacgagg	acttggactt	ccttacgaaa	tggactgatt	6840
ttaagtgcct	tgtttctgcg	tccaacatga	ggaatgcagc	aggccaattc	atcgaggctg	6900
cctatgcaaa	agcacttaga	attgaacttg	cccagttggg	gcaggttgat	aaggttcgag	6960
gtactttggc	caagcttgag	gcttttgctg	ataccgtggc	accccaactc	tcgcccgggtg	7020
acattgttgt	tgctcttggc	cacacgcctg	ttggcagcat	cttcgaccta	aaggttgggtg	7080
gtaccaagca	tactctccaa	gtcattgaga	ccagagtcct	tgccgggtcc	aaaatgaccg	7140
tggcgcgcgt	cgttgacca	acccccacgc	ccccaccgcg	acccgtgccc	atccccctcc	7200
caccgaaagt	tctagagaat	ggtcccaacg	cctgggggga	tggggaccgt	ttgaataaga	7260
agaagaggcg	taggatggaa	accgtcggca	tctttgtcat	gggtgggaag	aagtaccaga	7320
aattttggga	caagaattcc	ggtgatgtgt	tttacgagga	ggtccatgac	aacacagatg	7380
cgtgggagtg	cctcagagtt	ggtgaccctg	ccgactttaa	ccctgagaag	ggaactctgt	7440
gtgggcatac	tactattgaa	gataaggatt	acaaagtcta	cgcctcccca	tctggcaaga	7500
agttcctggg	ccccgtcaac	ccagagagcg	gaagagccca	atgggaagct	gcaaagcttt	7560
ccgtggagca	ggcccttggc	atgatgaatg	tcgacgggtg	actgacggcc	aaagaagtgg	7620
agaaactgaa	aagaataatt	gacaaacttc	agggcctgac	taaggagcag	tgtttaaact	7680
gctagccgcc	agcggcttga	cccgtgtgtg	tcgcggcgcc	ttggttgta	ctgagacagc	7740

-continued

ggtaaaaata	gtcaaatttc	acaaccggac	tttcacccta	gggcctgtga	atttaaaagt	7800
ggccagtga	gttgagctga	aagacgcggt	cgagcacaa	caacaccg	ttgcaagacc	7860
ggttgacggt	ggtggtgtgc	tctgcgttc	cgcagttcct	tcgcttatag	atgtcctgat	7920
ctccggtgct	gacgccttc	ctaagttact	cgctcgtcac	gggcgggga	acactgggat	7980
cgatggcacg	ctttgggact	ttgaggccga	ggccacaaa	gaggaaattg	cgctcagtgc	8040
gcaaataata	caggcttgtg	acattaggcg	cggtgacgca	cctgaaattg	gtctccctta	8100
caagctgtac	cctgttaggg	gcaaccctga	gcgggtaaaa	ggagttttac	agaatacaag	8160
gtttggagac	ataccttaca	aaacccccag	tgacactggg	agcccagtgc	acgcggctgc	8220
ctgcctcacg	cccaatgcca	ctccggtgac	tgatgggcgc	tccgtcttgg	ctactaccat	8280
gcctccggt	tttgaattgt	atgtaccgac	cattccagcg	tctgtccttg	attatcttga	8340
ctctaggcct	gactgcccc	aacagttgac	agagcacggc	tgtgaggatg	ccgcattgag	8400
agacctctcc	aagtatgact	tgtccacca	aggctttgtt	ttacctgggg	ttcttcgcct	8460
tgtgcgtaag	tacctgtttg	cccacgtggg	taagtgcgcg	cccgttcac	ggccttcac	8520
ttacctgcc	aagaattcta	tggctggaat	aatgggaac	aggtttccaa	ccaaggacat	8580
tcagagcgtc	cccgaaatcg	acgttctgtg	cgcacaggcc	gtgcgagaaa	actggcaaac	8640
tgttaccct	tgtaccctca	agaaacagta	ttgtgggaag	aagaagacta	ggacaatact	8700
cggcaccaat	aatttcattg	cgttgggcca	ccgggcagcg	ttgagtgggtg	tcaccagggtg	8760
cttcatgaaa	aaggcgttta	actcgcccat	cgcctcggg	aaaaacaaat	ttaaggagct	8820
acagactccg	atcttaggca	ggtgccttga	agctgatctt	gcacctctgtg	atcgatccac	8880
acctgcaatt	gtccgctggt	ttgccgccaa	ccttctttat	gaacttgcct	gtgctgaaga	8940
gcacctaccg	tcgtacgtgc	tgaactgctg	ccatgacct	ttggtcacgc	agtcgggcgc	9000
agtgactaag	aggggtggcc	tgtcgtcttg	cgaccgcatc	acttctgtgt	ctaacaccat	9060
ttacagcttg	gtgatatatg	cacagcacat	ggtgcttagt	tactttaaaa	gtggtcacc	9120
tcatggcctt	ctgttcctac	aagaccagct	gaagtctgag	gacatgctca	aagtccaacc	9180
cctgatcgtc	tattcgga	acctcgctg	gtatgccgaa	tctccacca	tgccgaacta	9240
ccactgggtg	gtcgaaatc	tgaatttgat	gctgggtttt	cagacggacc	caaagaagac	9300
agccataacg	gactcgccat	catttctagg	ctgtaggata	ataaatggac	gccagctagt	9360
ccccaaccgt	gacaggatcc	tcggggccct	cgttaccat	atgaaggcaa	gcaatgtttc	9420
tgaatactac	gccgcgcg	ctgcaatact	catggacagc	tgtgcttgtt	tagagtatga	9480
tctgaatgg	tttgaagagc	ttgtggttgg	gatagcgag	tgccgccgca	aggacggcta	9540
cagctttccc	ggcccgccgt	tcttcttctg	catgtgggaa	aaactcagat	ccaatcatga	9600
ggggaagaag	tccagaatgt	gcgggtattg	cggggccccg	gctccgtacg	ccactgcctg	9660
tggcctcgac	gtctgtat	accacacca	cttccaccag	cattgtccag	tcacaatctg	9720
gtgtggccac	ccggtggtt	ctggttcttg	tagtgagtgc	aaaccccccc	tagggaaagg	9780
cacaagccct	ctagatgagg	tgttagaaca	agtcctgtat	aagcctccac	ggactgtaat	9840
catgcatgtg	gagcagggtc	tcacccctct	tgaccaggc	agataccaga	ctcgccgcgg	9900
attagtctcc	gttaggcgtg	gcacagagg	aatgaagtt	gacctaccag	acggtgatta	9960
tgctagcacc	gccctactcc	ccacttgtaa	agagatcaac	atggtcgctg	tcgctctaa	10020
tgtgttgccg	agcaggttca	tcacgggtcc	gcccgggtgct	gggaaaacat	actggctcct	10080

-continued

tcagcaggtc	caggatggtg	atgtcattta	cacaccgact	caccagacca	tgctcgacat	10140
gattagggtc	ttggggacgt	gccggttcaa	cgtcccagca	ggtacaacgc	tgcaattccc	10200
tgccccctcc	cgtaccggcc	cgtgggttcg	catcctagcc	ggcggttggt	gtcctggtaa	10260
gaattccttc	ctggatgaag	cagcgtattg	taatcacctt	gatgtcttga	ggctccttag	10320
caaaaccacc	ctcacctgtc	tgggagactt	caaacaactc	caccagtggt	gttttgattc	10380
tcattgctat	gtttttgaca	tcatgcctca	gaccagttg	aagaccatct	ggagattcgg	10440
acagaacatc	tgtgatgcca	tccaaccaga	ttacagggac	aaacttgtgt	ccatgggtcaa	10500
cacaacccgt	gtaacctacg	tggaaaaacc	tgtcaagtat	gggcaagtcc	tcacccctta	10560
ccacagggac	cgagaggacg	gcgccatcac	aattgactcc	agtcaaggcg	ccacatttga	10620
tgtggtcaca	ctgcatttgc	ccactaaaga	ttcactcaac	aggcaaagag	cccttgttgc	10680
tatcaccagg	gcaagacatg	ctatctttgt	gtatgaccca	cacaggcaat	tgagagcat	10740
gtttgatctt	cctgcgaagg	gcacacccgt	caacctcgca	gtgcaccgtg	atgagcagct	10800
gacgtactg	gatagaaata	ataaagaatg	cacagttgct	caggctctag	gcaacggaga	10860
taaatttagg	gccaccgaca	agcgcgttgt	agattctctc	cgcgccattt	gtgctgatct	10920
ggaagggtcg	agctctccgc	tccccaaggt	cgcacacaac	ttgggatttt	atttctcacc	10980
tgatttgaca	cagtttgcta	aactcccggt	agaccttgca	ccccactggc	ccgtgggtgac	11040
aacccagAAC	aatgaaaagt	ggccggatcg	gctggttgcc	agccttcgcc	ctgtccataa	11100
gtatagccgt	gcgtgcattg	gtgccggcta	tatggtgggc	ccctcggtgt	ttctaggcac	11160
ccctggggtc	gtgtcatact	acctcacaaa	atttgtcaag	ggcgaggctc	aagtgcttcc	11220
ggagacagtc	ttcagcaccg	gccgaattga	ggtggattgc	cgaggagtac	ttgatgacag	11280
ggagcgagaa	gttgctgagt	ccctcccaca	tgcttccatt	ggcgacgtca	aaggcaccac	11340
cgttggggga	tgtcatcatg	tcacctccaa	ataccttcog	cgttcccttc	ccaaggaatc	11400
agtcgcggta	gtcgggggtt	cgagccccgg	gaaagccgca	aaagcagtgt	gcacattgac	11460
ggatgtgtac	ctcccagacc	ttgaggccta	cctccaccca	gagactcagt	ccaagtgctg	11520
gaaagtatat	ttggacttca	aggaagttcg	actgatggtc	tggaaagaca	agacggccta	11580
tttccaactt	gaaggccgct	atttcacctg	gtatcagctt	gcaagctacg	cctcgtagat	11640
ccgtgttcc	gtcaactcca	cgggtgtatc	ggacccctgc	atgggccctg	ccctttgcaa	11700
cagaagagtt	gtcgggtcca	cccatggggg	agctgacctc	gcagtcaccc	cttatgatta	11760
cgggtgctaaa	atcatcttgt	ctagcgctta	ccatggtgaa	atgcctcctg	gatacaagat	11820
tctggcgtgc	gcggagttct	cgtctgacga	ccagtcgaag	tacaaacaca	cctgggggtt	11880
tgaatcggat	acagcgtatc	tgtatgagtt	caccggaaac	ggtgaggact	gggaggatta	11940
caatgatgcg	tttcgtgcgc	gccagaaagg	gaaaatttat	aaggccactg	ctaccagcat	12000
gaagttttat	tttcccccg	gccccgtcat	tgaaccaact	ttaggcctga	attgaaatga	12060
aatggggctc	atacaaagcc	tcttcgacaa	aattggccag	ctttttgtgg	atgctttcac	12120
ggaatttttg	gtgtccattg	ttgatatcat	catatttttg	gccattttgt	ttggcttcac	12180
catcgccggt	tggctgggtg	tcttttgcat	cagattgggt	tgctccgcgg	tattccgtgc	12240
gcgccctgcc	attcaccttg	agcaattaca	gaagatccta	tgaggccttt	ctttctcagt	12300
gccgggtgga	cattcccacc	tggggggtaa	aacacccttt	ggggatgttt	tggcaccata	12360
aggtgtcaac	cctgattgat	gaaatgggtg	cgcgtcgaat	gtaccgcata	atggaaaaag	12420
cagggcaagc	tgcttgaaa	caggtgggtg	gcgaggctac	gctgtctcgc	attagtagtt	12480

-continued

tggatgtggt	ggctcathtt	caacatcttg	ccgccattga	agccgagacc	tgtaaattatt	12540
tggtttctcg	actgcccattg	ctacacaacc	tgcgcatgac	agggtcaaatt	gtaaccatag	12600
tgtataatag	cacttttaaatt	caggtgtttg	ctattttttcc	aacccttggt	tcccggccaa	12660
agcttcatga	ttttcagcaa	tggttaattag	ctgtacattc	ctccatattt	tcctctgttg	12720
cagcttcttg	tactcttttt	gttgtgctgt	ggttgccgggt	tccaatgcta	cgtactgttt	12780
ttggtttccg	ctggttagggt	gcaatttttc	tttcgaactc	atgggtgaatt	acacgggtgtg	12840
tccaccttgc	ctcaccggac	aagcagccgc	tgaggctcct	gaaccgggta	ggtctctttg	12900
gtgcaggata	gggcatgacc	gatgtgggga	ggacgatcac	gacgaactgg	ggttcatgggt	12960
tccgcctggc	ctctccagcg	aaagccactt	gaccagtgtt	tacgcctgggt	tggcggttcc	13020
gtccttcagc	tacacggccc	agttccatcc	cgagatattt	gggataggga	acgtgagtga	13080
agtttatgtt	gacatcaagc	accaattcat	ctgcgcggtt	catgacgggc	agaacaccac	13140
cttgccctcg	catgacaata	tttcagccgt	atttcagacc	tactatcaac	atcagggtcga	13200
cggcggcaat	tggtttcacc	tagaatggct	gcgtcccttc	ttttctctct	ggttggtttt	13260
aaatgtttcg	tggtttctca	ggcggtccgc	tgcaagccat	gtttcagttc	gagtctttca	13320
gacatcaaaa	ccaacactac	cgcagcatca	ggctttgttg	tcctccagga	catcagctgc	13380
cttaggcattg	gcgactcgtc	ctttccgacg	attcgcaaaa	gctctcaatg	ccgcacggcg	13440
atagggacac	ctgtgtatat	caccatcaca	gccaatgtga	cagatgagaa	ttacttacat	13500
tcttctgac	tcctcatgct	ttcttcttgc	cttttctatg	cttctgagat	gagtgaagaa	13560
ggattcaagg	tggtatttgg	caatgtgtca	ggcatcggtg	ctgtgtgtgt	caactttacc	13620
agctacgtcc	aacatgtcaa	agagtttacc	caacgctcct	tggtgggtcga	tcattgtcgg	13680
ctgcttcatt	tcattgacac	tgagaccatt	agggtgggcaa	ccgttttagc	ctgtcttttt	13740
gccatccctac	tggtcaattt	aatgttcaag	tatgttgggg	aaatgcttga	ccgcgggctg	13800
ttgctcgcga	ttgctttctt	tgtgggtgat	cgtgccgttc	tggtttgctg	tgctcggcaa	13860
cgcacaacagc	agcagcagct	ctcattttca	gttgatttat	aacttgacgc	tatgtgagct	13920
gaatggcaca	gattggctgg	caggaaaatt	tgattgggca	gtggagactt	ttgtcatctt	13980
tcccggtgtg	actcacattg	tttcttatgg	tgcaactcac	accagccatt	tccttgacac	14040
agttgggtctg	gttactgtgt	ccaccgcccg	gttttatcac	gggcgggtatg	tcttgagtag	14100
catctacgcg	gtctgtgctc	tggtgcggtt	gatttgcttc	gttattaggc	ttgcgaagaa	14160
ctgcatgtcc	tggtcgctact	cttgtaccag	atataccaac	ttccttcttg	acactaaggg	14220
cagactctat	cgttggcggt	cgcctgttat	catagaaaaa	gggggttaagg	ttgaggtcga	14280
aggatcatctg	atcgacctca	aaagagttgt	gcttgatggg	tccgtggcaa	cccccttaac	14340
cagagtttca	gcggaacaat	ggggtcgtct	ctagacgact	tttgccatga	tagcacggct	14400
ccacaaaagg	tgcttttggc	gttttccatt	acctacacgc	cagtaatgat	atatgtctca	14460
aaggtaagtc	gcggccgact	gctagggctt	ctgcaccttt	tgatctttct	gaattgtgct	14520
tttaccttcg	ggtacatgac	attcgcgcac	tttcagagca	caaattagggt	cgcgctcgct	14580
atggggagcag	tagttgcact	tctttggggg	gtgtactcag	ccatagaaac	ctggaaattc	14640
atcacctcca	gatgccgttt	gtgcttgcta	ggccgcaagt	acattctggc	ccctgcccac	14700
cacgtcgaaa	gtgccgcggg	ctttcatccg	attgcggcaa	atgataacca	cgcatttgct	14760
gtccggcgct	ccggctccat	tacggttaac	ggcacattgg	tgcccggtt	gaaaagcctc	14820

-continued

```

gtgttgggtg gcagaaaagc tgttaaaccg ggagtggtaa accttgtcaa atatgccaaa 14880
taacaacggc aagcagcaaa agaaaaagaa ggggaatggc cagccagtca accagctgtg 14940
ccagatgctg ggtaaaatca tcgcccagca aaaccagtcc agaggcaagg gaccgggctc 15000
taagaagaaa tccccggaga agccccatct tctctagcg accgaagatg acgtcaggca 15060
tcacttcacc cctggtgagc ggcaattgtg tctgtcgtcg atccagactg cctttaacca 15120
gggcgctgga acttgtacct tgtcagattc agggaggata agttacactg tggagttag 15180
tttgccgacg catcactg tgccctgat ccgcgtcaca gcatcacct cagcatgatg 15240
ggctggcatt ctttaggcac ctccagtgtc gaattggaag aatgtgtgtt ggatggcact 15300
gattgacatt gtgcctctaa gtcacctatt caattagggc gaccgtgtgg gggtaaaatt 15360
taattggcga gaaccatgcg gccgcaatta aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 15420
aaaaaaaaa aaaaaaaaaa aaaa 15444

```

<210> SEQ ID NO 5

<211> LENGTH: 15450

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: P129 passage 0 complete genome (15,450 nt) full length parental virus

<400> SEQUENCE: 5

```

atgacgtata ggtgttggct ctatgccacg gcatttgtat tgtcaggagc tgtgaccatt 60
ggcacagccc aaaacttgct gcacggaaaa cgcccttctg tgacagcctt cttcagggga 120
gcttaggggt ctgtccctag caccttgctt ctggagtgtc actgctttac ggtctctcca 180
cccctttaac catgtctggg atacttgatc ggtgcacgtg ccccccaat gccagggtgt 240
ttatggcgga gggccaagtc tactgcacac gatgtctcag tgcacggtct ctcttctctc 300
tgaatctcca agttctgag cttgggggtg tgggcctatt ttataggccc gaagagccac 360
tccggtggac gttgccacgt gcattcccca ctgtcgagtg ctcccccgcc ggggcctgct 420
ggctttctgc gatctttcca attgcacgaa tgaccagtgg aaacctgaac tttcaacaaa 480
gaatggtgct ggttgacgct gagatttaca gagccggcca actcaccctt gcagttttga 540
aggctctaca agtttatgaa cgggggtgtc gctggtacct cattgtcgga cctgtccctg 600
gagtggccgt tcacgccaac tccctacatg tgagtgaaca acctttcccg ggagcaactc 660
atgtgttaac caacctaccg ctcccgca gaagccaagcc tgaagacttt tgcccttttg 720
agtgtgctat ggctgacgtc tatgacatta gccatgacgc cgtcatgtat gtggccagag 780
ggaaagtctc ctgggcccct cgtggcgggg atgaagtga aattgaaacc gtccccgaag 840
agttgaagtt gattgcgaac cgactccaca tctccttccc gccccaccac gcagtggaca 900
tgtctgagtt tgccttcata gcccctggga gtggtgtctc cttgcgggtc gagcaccaac 960
acggctgcct tcccgtgat actgtccctg aagggaactg ctggtggtgc ttgtttgact 1020
tgctcccacc ggaagttcag aataaagaaa ttcgcctgtc taaccaattt ggctatcaaa 1080
ccaagcatgg tgtccctggc aagtacctac agcggaggct gcaagttaat ggtctccgag 1140
cagtgactga tacagatgga cctattgtcg tacagtactt ctctgttagg gagagttaga 1200
tccgccactt cagactggcg gaagaacctc gcctccctgg gtttgaagac ctctcagaa 1260
taagggtaga gcctaatacg tcgccattgg gtggcaaggg tgaaaaaatc ttccggtttg 1320
gcagtcacaa gtggtacggt gctggaaaga gagcaaggag agcacgctct ggtgcgactg 1380

```

-continued

ccacggctgc	tcaccgcgct	ttgcccgctc	gcgaagccca	gcaggccaag	aagctcgagg	1440
ttgccagcgc	caacagggct	gagcatctca	agtactattc	cccgcctgcc	gacgggaact	1500
gtggttggca	ctgcatttcc	gccattacca	accggatggg	gaattccaaa	tttgaaacca	1560
ctcttcccga	gagagtgaga	ccttcagatg	actgggctac	tgacgaggat	cttgtgaatg	1620
ccatccaaat	cctcaggctc	cccgcggcct	tggacaggaa	cgggtgcttg	gctggcgcca	1680
agtacgtgct	caagctggaa	ggtagagcact	ggaccgtctc	tgtgaccctc	gggatgaccc	1740
cttctttgct	cccccttgaa	tgtgttcagg	gttgttgtga	gcatacgagc	ggccttggtt	1800
ttccagatgt	ggtcgaagtt	tccggatttg	accctgcctg	ccttgaccga	cttgctgaga	1860
taatgcactt	gcctagcagt	gtcatcccag	ctgctctggc	cgagatgtcc	gacgacttca	1920
atcgtctggc	ttccccggcc	gccactgtgt	ggactgtttc	gcaattcttt	gcccgccaca	1980
gaggaggaga	gcacctcgac	cagggtgtgt	tagggaaaat	tatcaacctt	tgtcagggtga	2040
ttgaggaatg	ctgctgttcc	cggaacaaaag	ccaaccgggc	taccccgga	gaggttgctg	2100
caaaaattga	ccagtacctc	cgtgggtgcag	caagccttgg	agaatgcttg	gccaaagcttg	2160
agatggctcg	cccgcgcgagc	gcgatggaca	cctcctttga	ttggaatgtt	gtgcttctctg	2220
gggttgagac	ggcgaatcag	acaaccaaac	agctccatgt	caaccagtgc	cgcgctctgg	2280
ttcctgtcgt	gactcaagag	cctttggaca	gagactcagt	ccctctgacc	gccttctcgc	2340
tgtccaattg	ctactaccct	gcacaagggtg	acgaggtccg	tcaccgtgag	aggctaaact	2400
ccgtgctctc	taagttggag	ggggttggtc	gtgaggaata	tgggctcacg	ccaactggac	2460
ctggcccgcg	accgcgactg	ccgaacgggc	tcgacgagct	taaagaccag	atggaggagg	2520
atctgctgaa	attagtcaac	gcccaggcaa	cttcagaaat	gatggccttg	gcagccgagc	2580
aggttgatct	gaaagcttgg	gtcaaaaatt	accacgggtg	gacaccgcca	ccccctccac	2640
caagagttca	gcctcgaaaa	acgaagtctg	tcaagagctt	gctagagaac	aagcctgtcc	2700
ctgctccgcg	caggaagggtc	agatctgatt	gtggcagccc	gattttgatg	ggcgacaatg	2760
ttcctaacgg	ttgggaagat	tgcactgttg	gtggtccctc	tgacctttcg	gcaccatccg	2820
agccgatgac	acctctgagt	gagcctgtac	ttatttccag	gccagtgaca	tctttgagtg	2880
tgccggcccc	agttcctgca	cgcgctagag	ctgtgtcccg	accgatgacg	ccctcgagtg	2940
agccaatttt	tgtgtctgca	ctgcgacaca	aatttcagca	ggtggaaaaa	gcaaactctgg	3000
cggcagcagc	gccgatgtac	caggacgaac	ccttagattt	gtctgcatcc	tcacagactg	3060
aatatgaggc	ttctccccta	acaccaccgc	agaacgtggg	cattctggag	gtaagggggc	3120
aagaagctga	ggaagtcttg	agtgaatctc	cggatattct	gaatgacacc	aacctgcac	3180
ctgtgtcatc	aagcagctcc	ctgtcaagtg	ttaggatcac	acgccccaaa	tactcagctc	3240
aagccattat	cgactcgggc	gggccctgca	gtgggcacct	ccaaaggga	aaagaagcat	3300
gcctccgcat	catgcgtgag	gcttgtgatg	cggccaagct	tagtgaccct	gccacgcagg	3360
aatggctttc	tcgcatgtgg	gataggggtg	acatgctgac	ttggcgcaac	acgtctgctt	3420
accaggcggt	tcgcacctta	gatggcaggt	ttgggtttct	cccaaagatg	atactcgaga	3480
cgcgcgcgcc	ctaccctgtg	gggtttgtga	tgttgccctc	cacctctgca	ccttccgtga	3540
gtgcagagag	cgaccttacc	atcggttcag	tcgccactga	agatattcca	cgcacctcgc	3600
ggaaaataga	aaataccggt	gagatgatca	accagggacc	cttggcatcc	tctgaggaag	3660
aaccggtata	caaccaacct	gcccagact	cccggatatc	gtcgcggggg	tctgacgaga	3720
gcacagcagc	tccgtccgca	ggtacaggtg	gcgccggctt	atttactgat	ttgccacctt	3780

-continued

cagacggcgt	agatgcggac	ggtggggggc	cgttgcagac	ggtaagaaag	aaagctgaaa	3840
ggctcttctga	ccaattgagc	cgtcagggtt	ttaacctcgt	ctcccatctc	cctgttttct	3900
tctcacacct	cttcaaactc	gacagtgggt	attctccggg	tgattggggg	tttgcagctt	3960
ttactctatt	ttgcctcttt	ttgtgttaca	gctacccatt	cttcgggttc	gttccccctc	4020
tgggtgtatt	ttctgggtct	tctcggcgtg	tgcgcatggg	ggtttttggc	tgctggctgg	4080
cttttgctgt	tggcctgttc	aagcctgtgt	ccgacccagt	cggcactgct	tgtgagtttg	4140
actcgccaga	gtgtaggaac	gtccttcatt	cttttgagct	tctcaaacct	tgggaccctg	4200
ttcgcagcct	tgttgtgggc	cccgtcggtc	tcggtcttgc	cattcttggc	aggttactgg	4260
gcggggcacg	ctacatctgg	cattttttgc	ttaggcttgg	cattgttgca	gattgtatct	4320
tggctggagc	ttatgtgctt	tctcaaggta	ggtgtaaaaa	gtgctgggga	tcttgataaa	4380
gaactgctcc	taatgaaatc	gccttcaacg	tgttcccttt	tacacgtgcg	accaggtcgt	4440
cactcatcga	cctgtgcgat	cggttttgtg	cgccaaaagg	catggacccc	attttcctcg	4500
ccactgggtg	gcgtgggtgc	tggaccggcc	gaagtcccat	tgagcaacct	tctgaaaaac	4560
ccatcgcggt	cgcgcagttg	gatgaaaaga	ggattacggc	tagaactgtg	gtcgtcagc	4620
cttatgatcc	taatcaagcc	gtaaagtgtc	tgcggtgtgt	acaggcgggt	ggggcgatgg	4680
tggccgaggc	agtcccaaaa	gtggtcaaag	tttctgctat	tccattccga	gccccctttt	4740
ttcccaccgg	agtgaaagtt	gatcccagat	gcaggatcgt	ggttgacccc	gataactttta	4800
ctacagccct	ccggtctggt	tactctacca	caaacctcgt	ccttggtgtg	ggggactttg	4860
cccagctgaa	tggactaaaag	atcaggcaaa	tttccaagcc	ttcgggagga	ggcccacacc	4920
tcattgctgc	cctgcatgtt	gcctgctcga	tggcgttgca	catgcttgc	ggggtttatg	4980
taacttcagt	ggggtcttgc	ggtgcgggca	ccaacgatcc	atggtgcact	aatccgtttg	5040
ccgttcctgg	ctacggacca	ggctctctct	gcacgtccag	attgtgcac	tcccaacatg	5100
gccttaccct	gcccctgaca	gcacttgtgg	cgggattcgg	tcttcaggaa	atcgccctgg	5160
tcgttttgat	tttcgtttcc	atcggaggca	tggctcatag	gttgagttgt	aaggctgata	5220
tgctgtgcat	cttacttgca	atcgccagct	atgtttgggt	acccttacc	tggttgcttt	5280
gtgtgtttcc	ttgttggttg	cgtgggttct	ctttgcaccc	cctcaccatc	ctatggttgg	5340
tgtttttctt	gatttctgta	aatatgcctt	cgggaatctt	ggcgtgggtg	ttgttggttt	5400
ctctttggct	tttgggacgt	tatactaaca	ttgtcgggtc	tgtcaccccc	tatgatattc	5460
atcattacac	cagtggcccc	cgcgggtgtg	ccgccttagc	taccgcacca	gatggaacct	5520
acttggetgc	cgtccgccgc	gctgcgttga	ctggtcgcac	catgctgttc	accccgcttc	5580
agcttgggtc	ccttcttgag	ggcgctttca	gaactcgaaa	gccctcactg	aacaccgtca	5640
atgtggttgg	gtcctccatg	ggctctgggt	gagtgttcac	catcgacggg	aaaattaggt	5700
gcgtgactgc	cgcacatgtc	cttacgggta	attcggttag	ggtttcggga	gtcggcttca	5760
atcaaagtct	tgactttgat	gtgaaagggg	acttcgccat	agctgattgc	ccgaattggc	5820
aaggagctgc	tcccaagacc	caattctgcg	cggatggatg	gactggccgt	gcctattggc	5880
tgacatcctc	tggcgtcgaa	cccggtgtca	ttgggagtg	attcgcttc	tgttcaccg	5940
cgtgcggcga	ttccgggtcc	ccagtgatca	ccgaagctgg	tgagcttgtc	ggcggtcaca	6000
caggatcaaa	taaacaagga	ggtggcatcg	tcacgcgccc	ttcaggccag	ttttgtaacg	6060
tggcacccat	caagctgagc	gaattaagtg	aattctttgc	tggaccaag	gtcccgtcgc	6120

-continued

gtgatgtgaa	ggttggcagc	cacataatta	aagacacgtg	cgaagtacct	tcagatcttt	6180
gcgccttgct	tgctgcaaaa	cctgaactgg	agggaggcct	ctccaccgtc	caacttctgt	6240
gtgtgttttt	cctactgtgg	agaatgatgg	gacatgcctg	gacgccttg	gttgcgtgtg	6300
ggtttttcat	tctgaatgag	gttctcccag	ctgtcctggg	tcggagtgtt	ttctcctttg	6360
ggatgtttgt	gctatcttgg	ctcacaccat	ggctcgcgca	agttctgatg	atcaggcttc	6420
taacagcagc	tcttaacagg	aacagatggg	cacttgccct	ttacagcctt	ggcgcggtga	6480
ccggttttgt	cgcagatctt	gcggcaactc	aagggcaccc	gttgcaggca	gtaatgaatt	6540
tgagcaccta	tgccctcctg	cctcggatga	tggttgtgac	ctcaccagtc	ccagtgattg	6600
cgtgtgggtg	tgtgcaccta	cttgccatca	ttttgtactt	gtttaagtac	cgtggcctgc	6660
acaatgttct	tgttggtgat	ggagcgtttt	ctgcagcttt	cttcttgcca	tactttgccg	6720
agggaaagt	gagggaagg	gtgtcgcaat	cctgcggaat	gaatcatgag	tcattgactg	6780
gtgccctcgc	tatgagactc	aatgacgagg	acttggaact	ccttacgaaa	tggactgatt	6840
ttaagtgcct	tgtttctgcg	tccaacatga	ggaatgcagc	aggccaattc	atcgaggctg	6900
cctatgcaaa	agcacttaga	attgaacttg	cccagttggg	gcaggttgat	aaggttcgag	6960
gtactttggc	caagcttgag	gcttttgctg	ataccgtggc	accccaactc	tcgcccgggtg	7020
acattgttgt	tgctcttggc	catacgctg	ttggcagcat	cttcgaccta	aaggttggtg	7080
gtaccaagca	tactctccaa	gtcattgaga	ccagagtcc	tgccgggtcc	aaaatgaccg	7140
tggcgcgcgt	cgttgacca	acccccacgc	ccccacccgc	acccgtgccc	atccccctcc	7200
caccgaaagt	tctagagaat	ggtcccaacg	cctgggggga	tggggaccgt	ttgaataaga	7260
agaagaggcg	taggatggaa	accgtcggca	tctttgtcat	gggtgggaag	aagtaccaga	7320
aattttggga	caagaattcc	ggtgatgtgt	tttacgagga	ggtccatgac	aacacagatg	7380
cgtgggagtg	cctcagagtt	ggtgaccctg	ccgactttga	ccctgagaag	ggaactctgt	7440
gtgggcatac	tactattgaa	gataaggatt	acaaagtcta	cgcctcccca	tctggcaaga	7500
agttcctggg	ccccgtcaac	ccagagagcg	gaagagccca	atgggaagct	gcaaagcttt	7560
ccgtggagca	ggcccttggc	atgatgaatg	tcgacgggtg	actgacggcc	aaagaagtgg	7620
agaaactgaa	aagaataatt	gacaaacttc	agggcctgac	taaggagcag	tgtttaaact	7680
gctagccgcc	agcggcttga	cccgtgtgtg	tcgcggcggc	ttggttggtta	ctgagacagc	7740
ggtaaaaata	gtcaaatttc	acaaccggac	tttcacccta	gggcctgtga	atttaaaagt	7800
ggccagtgag	gttgagctga	aagacgcggg	cgagcacaac	caacaccgcg	ttgcaagacc	7860
ggttgacggg	ggtgttgtgc	tcctgcgttc	cgcagttcct	tcgcttatag	atgtcctgat	7920
ctccgggtgt	gacgcctctc	ctaagttact	cgtcgtcac	gggcggggga	acactgggat	7980
cgatggcacg	ctttgggact	ttgaggccga	ggccacaaaa	gaggaaattg	cactcagtgc	8040
gcaaataata	caggcttgtg	acattaggcg	cggcgacgca	cctgaaattg	gtctccctta	8100
caagctgtac	cctgttaggg	gcaaccctga	gcgggtaaaa	ggagttttac	agaatacaag	8160
gtttggagac	ataccttaca	aaacccccag	tgacactgga	agcccagtgc	acgcggctgc	8220
ctgcctcacg	cccaatgcca	ctccgggtgac	tgatgggcgc	tccgtcttgg	ctactaccat	8280
gccctccggg	tttgaattgt	atgtaccgac	cattccagcg	tctgtccttg	attatcttga	8340
ctctaggcct	gactgcccc	aacagttgac	agagcacggc	tgtgaggatg	ccgcattgag	8400
agacctctcc	aagtatgact	tgtccacca	aggctttgtt	ttacctgggg	ttcttcgcct	8460
tgtgcgtaag	tacctgtttg	cccacgtggg	taagtgcccg	cccgttcac	ggccttcac	8520

-continued

ttaccctgcc	aagaattcta	tggctggaat	aaatgggaac	aggtttccaa	ccaaggacat	8580
tcagagcgtc	cctgaaatcg	acgttctgtg	cgcacaggcc	gtgcgagaaa	actggcaaac	8640
tgttaccctt	tgtaccctca	agaaacagta	ttgtgggaag	aagaagacta	ggacaatact	8700
cggcaccaat	aatttcattg	cgttggccca	cggggcagcg	ttgagtgggtg	tcaccaggg	8760
cttcatgaaa	aaggcgttta	actcgcccat	cgccctcggg	aaaaacaaat	ttaaggagct	8820
acagactccg	gtcttaggca	ggtgccttga	agctgatctt	gcacctctgtg	atcgatccac	8880
acctgcaatt	gtccgctggt	ttgccgccaa	tcttctttat	gaacttgcct	gtgctgaaga	8940
gcacctaccg	tcgtacgtgc	tgaactgctg	ccatgaccta	ttggtcacgc	agtcgggcgc	9000
agtgactaag	aggggtggcc	tgtcgtctgg	cgacccgatc	acttctgtgt	ctaacaccat	9060
ttacagcttg	gtgatatatg	cacagcacat	ggtgcttagt	tactttaaaa	gtggtcacc	9120
tcattggcctt	ctgttcctac	aagaccagct	gaagttcgaa	gacatgctca	aagtccaacc	9180
cctgatcgtc	tattcggacg	acctcgtgct	gtatgccgaa	tctcccacca	tgccgaacta	9240
ccactgggtg	gtcgaacatc	tgaatttgat	gctgggtttt	cagacggacc	caaagaagac	9300
agccataacg	gactcgccat	catttctagg	ctgtaggata	ataaatggac	gccagctagt	9360
ccccaaaccgt	gacaggatcc	tcggggccct	cgcttaccat	atgaaggcaa	gcaatgtttc	9420
tgaatactac	gccgcggcgg	ctgcaatact	catggacagc	tgtgcttggt	tagagtatga	9480
tcctgaatgg	tttgaagagc	ttgtgggttg	gatagcgcag	tgcgcccga	aggacggcta	9540
cagctttccc	ggcccgcctg	tcttcttctc	catgtgggaa	aaactcagat	ccaatcatga	9600
ggggaagaag	tccagaatgt	gcgggtattg	cggggccccg	gctccgtacg	ccactgcctg	9660
tggcctcgac	gtctgtattt	accacaccca	cttccaccag	cattgtccag	tcataatctg	9720
gtgtggccac	ccggctggtt	ctgggttctt	tagtgagtgc	aaaccccccc	tagggaaagg	9780
cacaagccct	ctagatgagg	tgttagaaca	agtcccgtat	aagcctccac	ggactgtaat	9840
catgcatgtg	gagcagggtc	tcaccctctt	tgaccagggc	agataccaga	ctcgccgcgg	9900
attagtctcc	gttaggcgtg	gcacagagg	aatgaagtt	gacctaccag	acggtgatta	9960
tgctagcacc	gccctactcc	ccacttgtaa	agagatcaac	atggtcgctg	tcgcctctaa	10020
tgtgttgccg	agcaggttca	tcacgggtcc	gcccggtgct	gggaaaacat	actggctcct	10080
tcagcaggtc	caggatggtg	atgtcattta	cacaccgact	caccagacca	tgetcgacat	10140
gattagggct	ttggggacgt	gccggttcaa	cgtcccagca	ggtacaacgc	tgcaattccc	10200
tgccccctcc	cgtaccggcc	cgtgggttcg	catcctagcc	ggcggttggt	gtcctggtaa	10260
gaattccttc	ctggatgaag	cagcgtattg	taatcacctt	gatgtcttga	ggctccttag	10320
caaaaccacc	ctcacctgtc	tgggagactt	caaacaactc	caccagtggt	gttttgattc	10380
tcattgctat	gtttttgaca	tcatgcctca	gaccagttg	aagaccatct	ggagattcgg	10440
acagaacatc	tgtgatgcca	tccaaccaga	ttacagggac	aaacttgtgt	ccatgggtcaa	10500
cacaaccctg	gtaacctacg	tggaaaaacc	tgtcaagtat	gggcaagtcc	tcaccctta	10560
ccacagggac	cgagaggacg	gcgccatcac	aattgactcc	agtcaaggcg	ccacatttga	10620
tgtggttaca	ctgcatttgc	ccactaaaga	ttactcaac	aggcaaagag	cccttggtgc	10680
tatcaccagg	gcaagacatg	ctatctttgt	gtatgacca	cacaggcaat	tgcagagcat	10740
gtttgatctt	cctgcgaagg	gcacaccctg	caacctcgca	gtgcaccgtg	atgagcagct	10800
gatcgtactg	gatagaaata	ataaagaatg	cacagttgct	caggctctag	gcaacggaga	10860

-continued

taaatttagg	gccaccgaca	agcgcgttgt	agattctctc	cgcgccattt	gtgctgatct	10920
ggaagggtcg	agctctccgc	tccccaaggt	cgcacacaac	ttgggatttt	atttctcacc	10980
tgatttgaca	cagtttgcta	aactcccgg	agaccttgca	ccccactggc	ccgtgggtgac	11040
aacccagAAC	aatgaaaagt	ggccggatcg	gctggttgcc	agccttcgcc	ctgtccataa	11100
gtatagccgt	gcgtgcattg	gtgccggcta	tatgggtggc	ccctcgggtg	ttctaggcac	11160
ccctggggtc	gtgtcatact	acctcacaaa	atttgtcaag	ggcgaggctc	aagtgcttcc	11220
ggagacagtc	ttcagcaccg	gccgaattga	ggtggattgc	cgggggtatc	ttgatgacag	11280
ggagcgagaa	gttgctgagt	ccctcccaca	tgcttcatt	ggcgacgtca	aaggcaccac	11340
cgttggggga	tgtcatcatg	tcacctccaa	ataccttcgc	cgttccttc	ccaaggaatc	11400
agtcgcggta	gtcgggggtt	cgcgcggg	gaaagccgca	aaagcagtgt	gcacattgac	11460
ggatgtgtac	ctcccagacc	ttgaggccta	cctccaccca	gagactcagt	ctaagtgtcg	11520
gaaagtatat	ttggacttca	aggaagttcg	actgatggtc	tggaaagaca	agacggccta	11580
tttccaactt	gaaggccgct	atttcacctg	gtatcagctt	gcaagctacg	cctcgtagat	11640
ccgtgttctt	gtcaactcca	cgggtgtatc	ggacccctgc	atgggcccctg	ccctttgcaa	11700
cagaagagtt	gtcgggtcca	cccatggggg	agctgacctc	gcagtcaccc	cttatgatta	11760
cgggtgctaaa	atcatcttgt	ctagcgctta	ccatggtgaa	atgcctcctg	gatacaagat	11820
tctggcgtgc	gcggagtctt	cgtcgcagca	ccagtcgaag	tacaaacaca	cctgggggtt	11880
tgaatcggat	acagcgtatc	tgtatgagtt	catcggaaac	ggtgaggact	gggaggatta	11940
caatgatgcg	tttcgtgcgc	gccagaaagg	gaaaatttat	aaggccactg	ctaccagcat	12000
gaagttttat	tttcccccg	gccccgtcat	tgaaccaact	ttaggcctga	attgaaatga	12060
aatgggggtc	atacaaagcc	tcttcgacaa	aattggccag	ctttttgtgg	atgctttcac	12120
ggaatttttg	gtgtccattg	ttgatatcat	catatttttg	gccattttgt	ttggcttcac	12180
catcgccgg	tggctgggtg	tcttttgcat	cagattgggt	tgctccgcgg	tattccgtgc	12240
gcgccctgcc	attcaccttg	agcaattaca	gaagatccta	tgaggccctt	ctttctcagt	12300
gccgggtgga	cattcccacc	tggggggtaa	aacaccctt	ggggatgttt	tggcaccata	12360
aggtgtcaac	cctgattgat	gaaatgggtg	cgcgtcgaat	gtaccgcac	atggaaaaag	12420
cagggcaagc	tgcttggaag	caggtgggtg	gcgaggctac	gctgtctcgc	attagtagtt	12480
tggatgaggt	ggctcathtt	caacatcttg	ccgccattga	agccgagacc	tgtaaatatt	12540
tggcttctcg	actgcccatg	ctacacaacc	tgcgcatgac	aggggtcaat	gtaaccatag	12600
tgtataatag	cactttaaat	caggtgtttg	ctatttttcc	aacccttggt	tcccgcccaa	12660
agcttcatga	ttttcagcaa	tggtataatg	ctgtacattc	ctccatattt	tcctctgttg	12720
cagcttcttg	tactcttttt	gttgtgctgt	ggttgccggg	tccaatgcta	cgtactgttt	12780
ttggtttccg	ctggttaggg	gcaatttttc	tttcgaactc	atgggtgaatt	acacgggtgtg	12840
tccaccttgc	ctcaccgcg	aagcagccgc	tgaggccctt	gaaccgggta	ggtctctttg	12900
gtgcaggata	gggcatgacc	gatgtgggga	ggacgatcac	gacgaactag	ggttcatggt	12960
tccgcctggc	ctctccagcg	aaagccactt	gaccagtgtt	tacgcctggg	tggcgcttct	13020
gtccttcagc	tacacggccc	agttccatcc	cagatatatt	gggataggga	acgtgagtga	13080
agtttatggt	gacatcaagc	accaattcat	ctgcgcggtt	catgacgggc	agaacaccac	13140
cttgccctgc	catgacaata	tttcagccgt	atttcagacc	tactatcaac	atcaggctga	13200
cggcggaat	tggtttcacc	tagaatggct	gcgtcccttc	ttttctctt	ggttggtttt	13260

-continued

aaatgtttcg	tggtttctca	ggcggtcgcc	tgcaagccat	gtttcagttc	gagtctttca	13320
gacatcaaaa	ccaacactac	cgcagcatca	ggctttgttg	tcttccagga	catcagctgc	13380
cttaggcatg	gcgactcgtc	ctttccgacg	attcgcaaaa	gctctcaatg	ccgcacggcg	13440
atagggacac	ctgtgtatat	caccatcaca	gccaatgtga	cagatgagaa	ttacttacat	13500
tcttctgata	tctcatgct	ttcttcttgc	cttttctatg	cttctgagat	gagtgaaaag	13560
ggattcaagg	tggtgtttgg	caatgtgtca	ggcatcgtag	ctgtgtgtgt	caactttacc	13620
agctacgtcc	aacatgtcaa	agagtttacc	caacgctcct	tggtggtcga	tcatgtgcgg	13680
ctgcttcatt	tcatgacacc	tgagaccatg	aggtgggcaa	ccgttttagc	ctgtcttttt	13740
gccatcctac	tggaatttg	aatgttcaag	tatgttgggg	aaatgcttga	ccgcgggctg	13800
ttgctcgaga	ttgctttctt	tgtggtgtat	cgtgccgttc	tggtttgctg	tgctcgtcaa	13860
cgccaacaac	accagcagct	ctcattttca	gttgatttat	aacttgacgc	tatgtgagct	13920
gaatggcaca	gattggctgg	cagaaaaatt	tgattgggca	gtggagactt	ttgtcatctt	13980
tcccgtgttg	actcacattg	tttcttatgg	tgcaactcac	accagccatt	tccttgacac	14040
agttggctctg	gttactgtgt	ccaccgccgg	gttttatcac	gggcggtagt	tcttgagtag	14100
cgtctacgag	gtctgtgctc	tggtgcgtt	gatttgcttc	gtcattaggc	ttgcaagaa	14160
ctgcatgtcc	tggcgctact	cttgtagcag	atataccaac	ttccttctgg	acactaaggg	14220
cagactctat	cgttggcggg	cgcgcgttat	catagagaaa	gggggtaagg	ttgaggtcga	14280
aggtcacctg	atcgacctca	aaagagttgt	gcttgatggg	tccgtggcaa	cccccttaac	14340
cagagtttca	gcggaacaat	ggggtcgtct	ctagacgatt	tttgccatga	tagcacggct	14400
ccacaaaagg	tgcttttggc	gttttccatt	acctacacgc	cagtaatgat	atatgctcta	14460
aaggtaagtc	gcggccgact	gctagggctt	ctgcaccttt	tgatctttct	gaattgtgct	14520
tttaccttcg	ggtacatgac	attcgtgcac	tttcagagca	caaatagggt	cgcgctcact	14580
atgggagcag	tagttgcact	tctttggggg	gtgtactcag	ccatagaaac	ctggaaattc	14640
atcacctcca	gatgccgttt	gtgcttgcta	ggccgcaagt	acattctggc	ccctgcccac	14700
cacgtcgaaa	gtgccgcggg	ctttcatccg	attgcggcaa	atgataacca	cgcatttgct	14760
gtccggcgctc	ccggctccac	tacggttaac	ggcacattgg	tgcccggtt	gaaaagcctc	14820
gtgttggtg	gcagaaaagc	tgtaaacaag	ggagtggtaa	accttgtaa	atatgccaaa	14880
taacaacggc	aagcagcaaa	agaaaaagaa	ggggaatggc	cagccagtca	atcagctgtg	14940
ccagatgctg	ggtaaaatca	tcgccagca	aagccagtc	agaggcaagg	gaccgggcaa	15000
gaaaagtaag	aagaaaaacc	cggagaagcc	ccattttcct	ctagcgaccg	aagatgacgt	15060
caggcatcac	ttcaccctcg	gtgagcggca	attgtgtctg	tcgtcgatcc	agactgcctt	15120
taatcagggc	gctggaactt	gtaccctgtc	agattcaggg	aggataagtt	acactgtgga	15180
gtttagtttg	ccgacgcata	atactgtgcg	cctgatccgc	gtcacagcat	cacctcagc	15240
atgatgggct	ggcattcttt	aggcacctca	gtgtcagaat	tggaagagtg	tgtggtggat	15300
ggcactgatt	gacattgtgc	ctctaagtca	cctattcaat	tagggcgacc	gtgtgggggt	15360
aaaatttaat	tggcgagaac	catgcggccg	caattaaaaa	aaaaaaaaaa	aaaaaaaaaa	15420
aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa				15450

<210> SEQ ID NO 6
<211> LENGTH: 15450
<212> TYPE: DNA

-continued

```

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P129-PKC12-FL passage 52 complete genome
      (15,450 nt) full length vaccine virus

<400> SEQUENCE: 6

atgacgtata ggtggttgct ctatgccacg gcatttgtat tgtcaggagc tgtggccatt    60
ggcacagccc aaaacttgct gcacggaaaa cgcccttctg tgacagcctt cttcagggga    120
gcttaggggt ctgtccctag caccttgctt ctggagttgc actgctttac ggtctctcca    180
cccctttaac catgtctggg atacttgatc ggtgcacgtg ccccccaat gccagggtgt    240
ttatggcgga gggccaagtc tactgcacac gatgtctcag tgcacggtct ctcttctctc    300
tgaatctcca agttctgag cttggggtgc tgggcctatt ttataggccc gaagagccac    360
tccggtggac gttgccacgt gcattcccca ctgtcgagtg ctcccctgcc ggggcctgct    420
ggctttctgc gatctttcca attgcacgaa tgaccagtgg aaacctgaac tttcaacaaa    480
gaatggtgcg ggttgcagct gagatttaca gagccggcca actcaccctt gcagttttga    540
aggctctaca agtttatgaa cgggggttgc gctggtacct cattgtcgga cctgtccctg    600
gagtggccgt ttacgccaac tccctacatg tgagtgacaa acctttcccg ggagcaactc    660
atgtgttaac caacctaccg ctcccgcaga ggcccaagcc tgaagacttt tgcccttttg    720
agtgtgctat ggctaacgtc tatgacattg gccataacgc cgtcatgtat gtggccagag    780
ggaaagtctc ctgggcccct cgtggcgggg atgaagtga aattgaaacc gtccccgaag    840
agttgaagtt gattgcgaac cgactccaca tctccttccc gccccaccac gcagtggaca    900
tgtctgagtt tgccttcata gcccctggga gtggtgtctc cttgcgggtc gagcaccaac    960
acggctgctt tcccgtgat actgtccctg atgggaactg ctggtggtac ttgtttgact   1020
tgctcccacc ggaagttcag aataaagaaa ttcgccgtgc taaccaattt ggctatcaaa   1080
ccaagcatgg tgtccatggc aagtacctac agcggaggct gcaagttaat ggtctccgag   1140
cagtgactga tacagatgga cctattgtcg tacagtactt ctctgttagg gagagttgga   1200
tccgccactt cagactggcg gaagaaccta gcctccctgg gtttgaagac ctctcagaa   1260
taagggtaga gcctaatacg tcgccaatgg gtggcaaggg tgaaaaaatc ttccggtttg   1320
gcagtcacaa gtggtacggt gctggaaaga gagcaaggag agcacgctct ggtgcgactg   1380
ccacggctgc tcaccgcgtt ttgcccgctc gcgaagccca gcaggccaag aagctcgagg   1440
ttgccagcgc caacagggtt gagcatctca agtactatc cccgcctgcc gacgggaact   1500
gtggttgga ctgcatttcc gccattacca accggatggt gaattccaaa tttgaaacca   1560
ctcttcccga gagagtgaga ccttcagatg actgggctac tgacgaggat cttgtgaata   1620
ccatccaaat cctcaggctc cccgcggcct tggacaggaa cgggtgcttgt gctggcgcca   1680
agtacgtgct caagctggaa ggtgagcact ggaccgtctc tgtgaccctt gggatgacct   1740
cttctttgct ccccctgaa tgtgttcagg gttgttgtga gcataagagc ggtcttggtt   1800
tcccagacgt ggtcgaagtt tccggatttg accctgcttg tcttgaccga cttgctgaga   1860
taatgcactt gcctagcagt gtcacccag ctgctctggc cgagatgtcc gacgacttca   1920
atcgtctggc tccccggcc gccactgtgt ggactgtttc gcaattcttt gcccgccaca   1980
gaggaggaga gcatcctgac cagggtgtgt tagggaaaat tatcaacctt tgtcagggtga   2040
ttgaggaatg ctgctgttcc cggaacaaag ccaaccgggc taccceggaa gaggttgcg   2100
caaaagttga ccagtacctc cgtggtgcag caagccttgg agaatgcttg gccaaagcttg   2160

```

-continued

agagggctcg	cccgccgagc	gcgacggaca	cctcctttga	ttggaatgtt	gtgcttcctg	2220
gggttgagac	ggcgaatcag	acaaccaaac	agctccatgt	caaccagtgc	cgcgctctgg	2280
ttcctgtcgt	gactcaagag	cctttggaca	gagactcggt	ccctctgacc	gccttctcgc	2340
tgtccaattg	ctactaccct	gcacaagggt	acgagggtccg	tcaccgtgag	aggctaaact	2400
ccttgctctc	taagttggag	ggggttgttc	gtgaggaata	tgggctcacg	ccaactggac	2460
ctggcccgcg	accgcactg	cgaacgggc	tcgacgagct	taaagaccag	atggaggagg	2520
atctgctgaa	attagtcaac	gcccaggcaa	cttcagaaat	gatggcctgg	gcagccgagc	2580
aggttgatct	aaaagcttgg	gtcaaaaatt	accacgggtg	gacaccgcca	ccccctccac	2640
caagagttca	gcctcgaaaa	acgaagtctg	tcaagagctt	gctagagaac	aagcctgtcc	2700
ctgctccgcg	caggaaggtc	agatctgatt	gtggcagccc	gattttgatg	ggcgacaatg	2760
ttcctaacgg	ttgggaagat	togactgttg	gtggccccct	tgatctttcg	gcaccatccg	2820
agccgatgac	acctctgagt	gagcctgtac	ttatttccag	gccagtgaca	tctttgagtg	2880
tgccggcccc	agttcctgca	ccgcgtagag	ctgtgtcccc	accgatgacg	ccctcgagtg	2940
agccaatttt	tgtgtctgca	ctgcgacaca	aatttcagca	ggtggaaaaa	gcaaactctgg	3000
cggcagcagc	gccgatgtgc	caggacgaac	ccttagatth	gtctgcatcc	tcacagactg	3060
aatatgaggc	ttcccccta	acaccaccgc	agaacgtggg	cattctggag	gtaagggggc	3120
aagaagctga	ggaagtcttg	agtgaatct	cggatattct	gaatgatacc	aacctgcac	3180
ctgtgtcatc	aagcagctcc	ctgtcaagtg	ttaagatcac	acgccccaaa	tactcagctc	3240
aagccattat	cgactcgggc	gggccctgca	gtgggcacct	ccaaaggga	aaagaagcat	3300
gcctccgcat	catgcgtgaa	gcttgtgatg	cggccaagct	tagtgaccct	gccacgcagg	3360
aatggctttc	tcgcatgtgg	gatagggtgg	acatgctgac	ttggcgcaac	acgtctgctt	3420
accaggcggt	tcgcacctta	gatggcaggt	ttgggtttct	cccaaagatg	atactcgaga	3480
cgcgcgcgcc	ctaccctgtg	gggtttgtga	tgttgctca	cacctctgca	ccttccgtga	3540
gtgcagagag	cgaccttacc	attggttcag	tcgccactga	agatattcca	cgcacctcgc	3600
ggaaaataga	aaataccggt	gagatgatca	accagggaac	cttggcatcc	tctgaggaag	3660
aaccggtata	caaccaacct	gccaaagact	cccggatata	gtcgcggggg	tctgacgaga	3720
gcacagcagc	tccgtccgcg	ggtacagggt	gcgcgggctt	atttactgat	ttgccacctt	3780
cagacggcgt	agatgcggac	ggtggggggc	cgttgacagc	ggtaagaaa	aaagctgaaa	3840
ggctcttcga	ccaattgagc	cgtcagggtt	ttaacctcgt	ctcccatctc	cctgttttct	3900
tctcacacct	cttcaaactc	gacagtgggt	attctccggg	tgattggggg	tttgcagctt	3960
ttactctatt	ttgcctcttt	ttgtgttaca	gctacccatt	cttcggtttc	gttccccctc	4020
tgggtgtatt	ttctgggtct	tctcggcgtg	tgccatggg	ggtttttggc	tgctggctgg	4080
cttttgctgt	tggcctgttc	aagcctgtgt	ccgacccagt	cggcactgct	tgtgagtttg	4140
actcgccaga	gtgcaggaa	gtccttcatt	cttttgagct	tctcaaacct	tgggaccttg	4200
ttcgcagcct	tgttggtggc	cccgtcggtc	tcggctcttg	cattcttggc	aagttactgg	4260
gcggggcacg	ctacatctgg	cattttttgc	ttaggcttgg	cattgttgca	gattgtatct	4320
tggctggagc	ttatgtgctt	tctcaaggta	ggtgtaaaaa	gtgctgggga	tcttgataaa	4380
gaactgctcc	taatgaaatc	gccttcaacg	tgttcccttt	tacacgtgcg	accaggctcg	4440
cactcatcga	cctgtgcgat	cggttttgtg	cgccaacagg	catggacccc	attttcctcg	4500
ccactgggtg	gcgtgggtgc	tggaccggcc	gaagtcccat	tgagcaaccc	tctgaaaaac	4560

-continued

ccatcgcggtt	cgcccagttg	gatgaaaaga	ggattacggc	tagaactgtg	gtcgctcagc	4620
cttatgatcc	taatcaagcc	gtgaagtgtc	tgcgggtgtt	acaggcgggg	ggggcgatgg	4680
tggccgaggg	agtcccaaaa	gtggccaaag	tttctgctat	tccattccga	gccccctttt	4740
ttcccaccgg	agtgaaagtt	gatcccagat	gcaggatcgt	ggttgacccc	gataactttta	4800
ctacagccct	ccggtctggt	tactctacca	caaacctcgt	ccttggtgtg	ggggactttg	4860
cccagctgaa	tggactaaaag	atcaggcaaa	tttccaagcc	ttcgggagga	ggcccacacc	4920
tcattgtgtc	cctgcatgtt	gcctgtctga	tggcggttga	catgcttgtc	gggggtttatg	4980
taacttcagt	gggggtcttg	ggtgccggca	ccaacgatcc	atggtgcact	aatccgtttg	5040
ccgttcctgg	ctacggacca	ggctctctct	gcacgtccag	attgtgcac	tcccaacatg	5100
gccttaccct	gcccctgaca	gcacttgtgg	cgggattcgg	tcttcaggaa	atcgccctgg	5160
tcgttttgat	tttctgttcc	atcggaggca	tggctcatag	gttgagttgt	aaggctgata	5220
tgtgtgtcat	tttacttgca	atcgccagct	atgtttgggt	accccttacc	tgggtgcttt	5280
gtgtgtttcc	ttgttggttg	cgtcggttct	ctttgcaccc	ccttaccatc	ctatggttgg	5340
tgtttttctt	gatttctgta	aatatgcctt	cgggaatctt	ggccgtggtg	ttattggttt	5400
ctctttgggt	tttgggacgt	tatactaaca	ttgtctggtc	tgtcaccccc	tatgatattc	5460
atcattacac	cagtggcccc	cgcggtgttg	ccgccttggc	taccgcacca	gatggaacct	5520
acttggtgtc	cgtccgccgc	gctgcgttga	ctggtcgcac	catgctgttc	accccgcttc	5580
agcttgggtc	ccttcttgag	ggcgctttca	gaactcgaaa	gccctcactg	aacaccgtca	5640
atgtgggttg	gtcctccatg	ggctctgggt	gagtgttcac	catcgacggg	aaaattaggt	5700
gcgtgactgc	cgcacatgtc	cttacgggta	attcggttag	ggtttccgga	gtcgggttca	5760
atcaaagtgt	tgactttgat	gtgaaagggg	acttcgccat	agctgattgc	ccgaattggc	5820
aaggagctgc	tcccaagacc	caattctgct	aggacggatg	gactggccgt	gcctattggc	5880
tgacatcctc	tggcgctgaa	cccgggtgta	ttgggaatgg	attcgccctc	tgccttcaccg	5940
cgtgcggcga	ttccgggtcc	ccagtgatca	ccgaagctgg	tgagattgtc	ggcggttcaca	6000
caggatcaaa	taaacaagga	ggtggcatcg	tcacgcgccc	ttcaggccag	ttttgtaacg	6060
tggcacccat	caagctgagc	gaattaagtg	aattctttgc	tggacccaag	gtcccgcctc	6120
gtgatgtgaa	ggttggcagc	cacataatta	aagacacgtg	cgaagtacct	tcagatcttt	6180
gcgccttgct	tgctgccaaa	cctgaactgg	agggaggcct	ctccaccgtc	caacttctgt	6240
gtgtgttttt	cctactgtgg	agaatgatgg	gacatgcctg	gacgcccttg	gttgcgtgtg	6300
ggtttttcat	tctgaatgag	gttctcccag	ctgtcctggg	tcggagtgtt	ttctcctttg	6360
ggatgtttgt	gctatcttgg	ctcacaccat	ggtctgcgca	agttctgatg	atcaggcttc	6420
taacagcagc	tcttaacagg	aacagatggg	cacttgccct	ttacagcctt	ggtgcgggtga	6480
ccggttttgt	cgcagatctt	gcggtaactc	aagggcaccc	gttgcaggca	gtaatgaatt	6540
tgagcaccta	tgccttcctg	cctcggtatg	tggttgtgac	ctcaccagtc	ccagtgattg	6600
cgtgtgggtg	tgtgcaccta	cttgccatca	ttttgtactt	gttcaagtac	cgcggcctgc	6660
acaatgttct	tgttggtgat	ggagcgtttt	ctgcagcttt	cttcttgcca	tactttgccg	6720
agggaaaagt	gagggaaagg	gtgtcgcaat	cctgcggaat	gaatcatgag	tcattgactg	6780
gtgccctcgc	tatgagactc	aatgacgagg	acttggaact	ccttacgaaa	tggactgatt	6840
ttaagtgtct	tgtttctgct	tccaacatga	ggaatgcagc	aggccaattc	atcgaggctg	6900

-continued

cctatgcaaa	agcacttaga	attgaacttg	cccagttggt	gcaggttgat	aaggttcgag	6960
gtactttggc	caagcttgag	gcttttgctg	ataccgtggc	accccaactc	tcgcccgggtg	7020
acattgttgt	tgctcttggc	catacgctg	ttggcagcat	cttcgaccta	aaggttggtg	7080
gtaccaagca	tactctccaa	gtcattgaga	ccagagtcc	tgccgggtcc	aaaatgaccg	7140
tggcgcggt	cgttgaccca	acccccacgc	ccccacccgc	acccgtgccc	atccccctcc	7200
caccgaaagt	tctagagaat	ggtcccaacg	cctgggggga	tggggaccgt	ttgaataaga	7260
agaagaggcg	taggatggaa	accgtcggca	tctttgtcat	gggtgggaag	aagtaccaga	7320
aattttggga	caagaattcc	ggtgatgtgt	tttacgagga	ggtccatgac	aacacagatg	7380
cgtgggagtg	cctcagagtt	ggtgaccctg	cgcactttaa	ccctgagaag	ggaactctgt	7440
gtgggcatac	tactattgaa	gataaggatt	acaaagtcta	cgcctcccca	tctggcaaga	7500
agttcctggt	ccccgtcaac	ccagagagcg	gaagagccca	atgggaagct	gcaaagcttt	7560
ccgtggagca	ggcccttggc	atgatgaatg	tcgacgggtga	actgacggcc	aaagaagtgg	7620
agaaactgaa	aagaataatt	gacaaacttc	agggccttac	taaggagcag	tgtttaaact	7680
gctagccgcc	agcggcttga	cccgtgtgtg	tcgcggcggc	ttggttggtta	ctgagacagc	7740
ggtaaaaata	gtcaaatttc	acaaccggac	tttcacccta	gggcctgtga	atttaaaagt	7800
ggccagtgag	gttgagctga	aagacgcggg	cgagcacaac	caacaccccg	ttgcaagacc	7860
ggttgacggg	ggtgttgtgc	tccgtgcgttc	cgcagttcct	tcgcttatag	atgtcctgat	7920
ctccggtgct	gacgcctctc	ctaagttact	cgcctgtcac	gggcggggga	acactgggat	7980
cgatggcacg	ctttgggact	ttgaggccga	ggccacccaa	gaggaaattg	cgcctcagtgc	8040
gcaaataata	caggcttgtg	acattaggcg	cggtagcgca	cctgaaattg	gtctccctta	8100
caagctgtac	cctgttaggg	gcaaccctga	gcgggtaaaa	ggagttttac	agaatacaag	8160
gtttggagac	ataccttaca	aaacccccag	tgacactggg	agcccagtgc	acgcggctgc	8220
ctgcctcacg	cccaatgcca	ctccggtgac	tgatggggcg	tccgtcttgg	ctactaccat	8280
gccctccggt	tttgaattgt	atgtaccgac	cattccagcg	tctgtccttg	attatcttga	8340
ctctaggcct	gactgcccc	aacagttgac	agagcacggc	tgtgaggatg	ccgcattgag	8400
agacctctcc	aagtatgact	tgtccacca	aggctttggt	ttacctgggg	ttcttcgcct	8460
tgtgcgtaag	tacctgtttg	cccacgtggg	taagtgcgg	cccgttcctc	ggccttcac	8520
ttaccctgcc	aagaattcta	tggctggaat	aatgggaac	aggtttccaa	ccaaggacat	8580
tcagagcgtc	cccgaaatcg	acgttctgtg	cgcacaggcc	gtgcgagaaa	actggcaaac	8640
tgttaccctt	tgtaccctca	agaaacagta	ttgtgggaag	aagaagacta	ggacaatact	8700
cggcaccaat	aatttcattg	cgttgggcca	ccgggcagcg	ttgagtgggt	tcaccaggg	8760
cttcatgaaa	aaggcgttta	actcgcccat	cgcctcggg	aaaaacaaat	ttaaggagct	8820
acagactccg	atcttaggca	ggtgccttga	agctgatctt	gcctcctgtg	atcgatccac	8880
acctgcaatt	gtccgctggt	ttgccgccaa	ccttctttat	gaacttgctt	gtgctgaaga	8940
gcacctaccg	tcgtacgtgc	tgaactgctg	ccatgaccta	ttggtcacgc	agtcgggcgc	9000
agtgactaag	aggggtggcc	tgtcgtctgg	cgacccgatc	acttctgtgt	ctaaccacat	9060
ttacagcttg	gtgatatatg	cacagcacat	ggtgcttagt	tactttaaaa	gtggtcaccc	9120
tcatggcctt	ctgttcctac	aagaccagct	gaagtctgag	gacatgctca	aagtccaacc	9180
cctgategtc	tattcggacg	acctcgtgct	gtatgccgaa	tctcccacca	tgcggaacta	9240
ccactggtgg	gtcgaacatc	tgaatttgat	gctgggtttt	cagacggacc	caaagaagac	9300

-continued

agccataacg gactcgccat catttctagg ctgtaggata ataaatggac gccagctagt	9360
ccccaacctg gacaggatcc tcgcgccctt cgcttaccat atgaaggcaa acaatgtttc	9420
tgaatactac gccgcggcgg ctgcaatact catggacagc tgtgcttggt tagagtatga	9480
tcctgaatgg tttgaagagc ttgtggttgg gatagcgcat tgcgcccga aggacggcta	9540
cagctttccc ggcccgcctg tcttcttgtc catgtgggaa aaactcagat ccaatcatga	9600
ggggaagaag tccagaatgt gcgggtattg cggggccctg gctccgtacg ccaactgcctg	9660
tggcctcgac gtctgtatct accacaccca cttccaccag cattgtccag tcacaatctg	9720
gtgtggccac ccggctggtt ctgggttctt tagtgagtgc aaaccccccc tagggaaagg	9780
cacaagccct ctagatgagg tgttagaaca agtcccgtat aagcctccac ggactgtaat	9840
catgcatgtg gagcagggtc tcacccctct tgacccaggc agataccaga ctgcgcgagg	9900
attagtctcc gttaggcgtg gcacagagg aaatgaagtt gacctaccag acggtgatta	9960
tgctagcacc gccctactcc ccacttgtaa agagatcaac atggctcgctg tcgcctctaa	10020
tgtgttgccc agcagggtca tcacgggtcc gcccggtgct gggaaaacat actggctcct	10080
tcagcaggtc caggatggtg atgtcattta cacaccgact caccagacca tgctcgacat	10140
gattagggtt ttggggacgt gccgggtcaa cgtcccagca ggtgcaacgc tgcaattccc	10200
tgccccctcc cgtaccggcc cgtgggttcg catcctagcc ggcggttggt gtcttggtaa	10260
gaattccttc ttggatgaag cagcgtattg taatcacctt gatgtcttga ggctccttag	10320
caaaaccacc ctcacctgtc tgggagactt caaacaactc caccagtggt gttttgattc	10380
tcattgctat gtttttgaca tcatgcctca gaccagttg aagaccatct ggagattcgg	10440
acagaacatc tgtgatgcca tccaaccaga ttacagggac aaacttgtgt ccatggtcaa	10500
cacaaccctg gtaaccacg tggaaaaacc tgtcaagtat gggcaagtcc tcacccctta	10560
ccacagggac cgagaggacg gcgccatcac aattgactcc agtcaaggcg ccacatttga	10620
tgtggtcaca ctgcatttgc ccactaaaga ttcactcaac aggcaaagag cccttggtgc	10680
tatcaccagg gcaagacatg ctatctttgt gtatgacca cacaggcaat tgacagacat	10740
gtttgatctt cctgcgaagg gcacaccctg caacctcgca gtgcaccgtg atgagcagct	10800
gatcgtagtg gatagaaata ataaagaatg cacagttgct caggctctag gcaacggaga	10860
taaatttagg gccaccgaca agcgcgttgt agattctctc cgcgccattt gtgctgatct	10920
ggaagggctg agctctccgc tccccagggt cgcacacaac ttgggatttt atttctcacc	10980
tgatttgaca cagtttgcta aactcccggt agaccttgca cccactggc ccgtggtgac	11040
aaccagaaac aatgaaaagt ggccggatcg gctggttgcc agccttcgcc ctgtccataa	11100
gtatagccgt gcgtgcattg gtgcccgtta tatggtgggc ccctcggtgt ttctaggcac	11160
ccctggggtc gtgtcatact acctcacaaa atttgtcaag ggcgaggctc aagtgcttcc	11220
ggagacagtc ttcagcaccg gccgaattga ggtggattgc cgggagtatc ttgatgacag	11280
ggagcgagaa gttgctgagt ccctcccaca tgccttcatt ggcgacgtca aaggcaccac	11340
cgttggggga tgtcatcatg tcacctcaa ataccttcgg cgcttccttc ccaaggaatc	11400
agtcgcggta gtcgggggtt cgagccccgg gaaagccgca aaagcagtgt gcacattgac	11460
ggatgtgtac ctcccagacc ttgaggccta cctccacca gagactcagt ccaagtgtctg	11520
gaaagtatat ttggacttca aggaagtctg actgatggtc tggaaagaca agacggccta	11580
tttccaactt gaaggccgct atttcacctg gtatcagctt gcaagctacg cctcgtacat	11640

-continued

ccgtgttcc	gtcaactcca	cgggtgtatct	ggacccctgc	atgggccctg	ccctttgcaa	11700
cagaagagtt	gtcgggtcca	cccattgggg	agctgacctc	gcagtcaccc	cttatgatta	11760
cggtgctaaa	atcatcttgt	ctagcgctta	ccatggtgaa	atgcctcctg	gatacaagat	11820
tctggcgtgc	gcggagttct	cgctcgacga	cccagtcaag	tacaaacaca	cctgggggtt	11880
tgaatcggat	acagcgtatc	tgtatgagtt	caccggaaac	ggtgaggact	gggaggatta	11940
caatgatgcg	tttcgtgcgc	gccagaaagg	gaaaatttat	aaggccactg	ctaccagcat	12000
gaagttttat	tttcccccg	gccccgtcat	tgaaccaact	ttaggcctga	attgaaatga	12060
aatggggtct	atacaaagcc	tcttcgacaa	aattggccag	ctttttgtgg	atgctttcac	12120
ggaatttttg	gtgtccattg	ttgatatcat	catatttttg	gccattttgt	ttggcttcac	12180
catcgccggt	tggctggtgg	tcttttgcac	cagattgggt	tgctccgcgg	tattccgtgc	12240
gcgccctgcc	attcaccctg	agcaattaca	gaagatccta	tgaggccttt	ctttctcagt	12300
gccgggtgga	cattcccacc	tggggggtaa	aacacccttt	ggggatgttt	tggcaccata	12360
aggtgtcaac	cctgattgat	gaaatgggtg	cgcgtcgaat	gtaccgcgtc	atggataaag	12420
cagggcaagc	tgcttggaag	caggtggtga	gcgaggttac	gctgtctcgc	attagtagtc	12480
tggatgtggt	ggctcatttt	caacatcttg	ccgccattga	agccgagacc	tgtaaataat	12540
tggcttctcg	actgcccatt	ctacacaacc	tgcgcatgac	aggggtcaa	gtaaccatag	12600
tgtataatag	cactttaaat	caggtgtttg	ctatttttcc	aacccttggt	tcccgcccaa	12660
agcttcatga	ttttcagcaa	tggttaatat	ctgtacattc	ctccatattt	tctctgtttg	12720
cagcttcttg	tactcttttt	gttgtgctgt	ggttgccggg	tccaatgcta	cgtactgttt	12780
ttggtttccg	ctggttaggg	gcaatttttc	tttcgaactc	atgggtgaat	acacgggtgtg	12840
tccaccttgc	ctcaccgcgc	aagcagccgc	tgaggctcct	gaaccgcgta	ggtctctttg	12900
gtgcaggata	gggcatgacc	gatgtgggga	ggacgatcac	gacgaactgg	ggttcatggg	12960
tccgcctggc	ctctccagcg	aaagccactt	gaccagtgtt	tacgcctggg	tggcggttcc	13020
gtccttcagc	tacacggccc	agttccatcc	cgagatatct	gggataggga	acgtgagtga	13080
agtttatggt	gacatcaagc	accaattcat	ctgcgccgtt	catgacgggc	agaacaccac	13140
cttgccctgc	catgacaata	tttcagccgt	atttcagacc	tactatcaac	atcagggtcga	13200
cggcggaat	tggtttcacc	tagaatggct	gcgtcccttc	ttttctctct	ggttggtttt	13260
aaatgtttcg	tggttttctc	ggcggttcgc	tgcaagccat	gtttcagttc	gagtctttca	13320
gacatcaaaa	ccaacactac	cgcagcatca	ggctttgttg	tcttcagga	catcagctgc	13380
cttaggcatt	gcgactcgtc	ctttccgacg	attcgcaaaa	gctctcaatg	ccgcacggcg	13440
atagggacac	ctgtgtatat	caccatcaca	gccaatgtga	cagatgagaa	ttacttacat	13500
tcttctgac	tctcatgct	ttcttcttgc	cttttctatg	cttctgagat	gagtgaagaa	13560
ggattcaagg	tggatatttg	caatgtgtca	ggcatcgtgg	ctgtgtgtgt	caactttacc	13620
agctacgtcc	aacatgtcaa	agagtttact	caacgctcct	tgggtggtcga	tcatgtgcgg	13680
ctgcttcatt	tcatgacacc	tgagaccatg	aggtgggcaa	ccgttttagc	ctgtcttttt	13740
gccatccctac	tggcaatttg	aatgttcaag	tatgttgggg	aaatgcttga	ccgcgggctg	13800
ttgctcgcga	ttgctttctt	tgtgggtgat	cgtgccgttc	tggtttgctg	tgctcggcaa	13860
cgccaacagc	agcagcagct	ctcattttca	gttgatttat	aacttgacgc	tatgtgagct	13920
gaatggcaca	gattggctgg	cagaaaaatt	tgattggggc	gtggagactt	ttgtcatctt	13980
tcccggtgtg	actcacattg	tttctatttg	tgcactcacc	accagccatt	tcttgacac	14040

-continued

agttggtctg gttactgtgt ccaccgccgg gttttatcac gggcggtatg tcttgagtag	14100
catctacggg gtctgtgctc tggetgcgtt gatttgcttc gttattaggc ttgcgaagaa	14160
ctgcatgtcc tggcgctact cttgtaccag atataccaac ttccttcttg acactaaggg	14220
cagactctat cgttggcggg cgcgcgttat catagaaaaa aggggtaagg ttgaggtcga	14280
aggatcatctg atcgacctca aaagagttgt gcttgatggg tccgtggcaa cccctttaac	14340
cagagtttca gcggaacaat ggggtcgtct ctagacgact tttgccatga tagcactgct	14400
ccacaaaagg tgcttttggc gttttccatt acctacacgc cagtaatgat atatgctcta	14460
aaggtaagtc gcggccgact gctagggctt ctgcaccttt tgatctttct gaattgtgct	14520
tttaccttcg ggtacatgac attcgcgcac tttcagagca caaatagggt cgcgctcgct	14580
atgggagcag tagttgact tctttggggg gtgtactcag ccatagaaac ctggaaattc	14640
atcacctcca gatgccgttt gtgcttgcta ggccgcaagt acattctggc ccctgcccac	14700
cacgtcgaaa gtgccgcggg ctttcatccg attgcggcaa atgataacca cgcatttgct	14760
gtccggcgct cgggctccat tacggttaac ggacattggg tgcccggggt gaaaagcctc	14820
gtgttggggt gcagaaaagc tgttaaacag ggagtggtaa accttgtaa atatgcaaaa	14880
taacaacggc aagcagcaaa agaaaaagaa ggggaatggc cagccagtca accagctgtg	14940
ccagatgctg ggtaaaatca tcgccagca aaaccagtc agaggcaagg gaccgggcaa	15000
gaaaagtaag aagaaaaacc cggagaagcc ccattttcct ctagcgaccg aagatgacgt	15060
caggcatcac ttcaccctg gtgagcggca attgtgtctg tcgtcgatcc agactgcctt	15120
taaccagggc gctggaactt gtaccctgtc agattcaggg aggataagtt aactgtgga	15180
gtttagtttg ccgacgcac atactgtgcg cctgatccgc gtcacagcat caccctcagc	15240
atgatggggt ggcattcttt aggcacctca gtgtcagaat tggaagaatg tgtgggtgat	15300
ggcactgatt gacattgtgc ctctaagtca cctattcaat tagggcgacc gtgtgggggt	15360
aaaatttaat tggcgagAAC catgcggccg caattaaaaa aaaaaaaaaa aaaaaaaaaa	15420
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa	15450
<210> SEQ ID NO 7	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 735, ORF1a: 182, Nsp1b: 2, passage 0 amino acid	
<400> SEQUENCE: 7	
Ala Met Ala Asp Val Tyr Asp	
1 5	
<210> SEQ ID NO 8	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 735, ORF1a: 182, Nsp1b: 2, passage 17 amino acid	
<400> SEQUENCE: 8	
Ala Met Ala Asn Val Tyr Asp	
1 5	
<210> SEQ ID NO 9	

-continued

<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 735, ORF1a: 182, Nsp1b: 2,
passage 52 amino acid

<400> SEQUENCE: 9

Ala Met Ala Asn Val Tyr Asp
1 5

<210> SEQ ID NO 10
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 756, ORF1a: 189, Nsp1b: 9,
passage 0 amino acid

<400> SEQUENCE: 10

Ile Ser His Asp Ala Val Met
1 5

<210> SEQ ID NO 11
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 756, ORF1a: 189, Nsp1b: 9,
passage 17 amino acid

<400> SEQUENCE: 11

Ile Gly His Asn Ala Val Met
1 5

<210> SEQ ID NO 12
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 756, ORF1a: 189, Nsp1b: 9,
passage 52 amino acid

<400> SEQUENCE: 12

Ile Gly His Asn Ala Val Met
1 5

<210> SEQ ID NO 13
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 992, ORF1a: 267, Nsp1b: 87,
passage 0 amino acid

<400> SEQUENCE: 13

Thr Val Pro Glu Gly Asn Cys
1 5

<210> SEQ ID NO 14
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 992, ORF1a: 267, Nsp1b: 87,
passage 17 amino acid

<400> SEQUENCE: 14

-continued

Thr Val Pro Asp Gly Asn Cys	
1	5
<210> SEQ ID NO 15	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 992, ORF1a: 267, Nsp1b: 87, passage 52 amino acid	
<400> SEQUENCE: 15	
Thr Val Pro Asp Gly Asn Cys	
1	5
<210> SEQ ID NO 16	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 1009, ORF1a: 273, Nsp1b: 93, passage 0 amino acid	
<400> SEQUENCE: 16	
Cys Trp Trp Cys Leu Phe Asp	
1	5
<210> SEQ ID NO 17	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 1009, ORF1a: 273, Nsp1b: 93, passage 17 amino acid	
<400> SEQUENCE: 17	
Cys Trp Trp Tyr Leu Phe Asp	
1	5
<210> SEQ ID NO 18	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 1009, ORF1a: 273, Nsp1b: 93, passage 52 amino acid	
<400> SEQUENCE: 18	
Cys Trp Trp Tyr Leu Phe Asp	
1	5
<210> SEQ ID NO 19	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 1096, ORF1a: 302, Nsp1b: 122, passage 0 amino acid	
<400> SEQUENCE: 19	
His Gly Val Pro Gly Lys Tyr	
1	5
<210> SEQ ID NO 20	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	

-continued

<223> OTHER INFORMATION: genome position 1096, ORF1a: 302, Nsp1b: 122, passage 17 amino acid	
<400> SEQUENCE: 20	
His Gly Val His Gly Lys Tyr	
1 5	
<210> SEQ ID NO 21	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 1096, ORF1a: 302, Nsp1b: 122, passage 52 amino acid	
<400> SEQUENCE: 21	
His Gly Val His Gly Lys Tyr	
1 5	
<210> SEQ ID NO 22	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 2106, ORF1a: 639, Nsp2: 256, passage 0 amino acid	
<400> SEQUENCE: 22	
Ala Ala Lys Ile Asp Gln Tyr	
1 5	
<210> SEQ ID NO 23	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 2106, ORF1a: 639, Nsp2: 256, passage 17 amino acid	
<400> SEQUENCE: 23	
Ala Ala Lys Val Asp Gln Tyr	
1 5	
<210> SEQ ID NO 24	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 2106, ORF1a: 639, Nsp2: 256, passage 52 amino acid	
<400> SEQUENCE: 24	
Ala Ala Lys Val Asp Gln Tyr	
1 5	
<210> SEQ ID NO 25	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 2185, ORF1a: 665, Nsp2: 282, passage 0 amino acid	
<400> SEQUENCE: 25	
Pro Ser Ala Met Asp Thr Ser	
1 5	

-continued

<210> SEQ ID NO 26
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 2185, ORF1a: 665, Nsp2: 282,
passage 17 amino acid

<400> SEQUENCE: 26

Pro Ser Ala Thr Asp Thr Ser
1 5

<210> SEQ ID NO 27
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 2185, ORF1a: 665, Nsp2: 282,
passage 52 amino acid

<400> SEQUENCE: 27

Pro Ser Ala Thr Asp Thr Ser
1 5

<210> SEQ ID NO 28
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 2403, ORF1a: 738, Nsp2: 355,
passage 0 amino acid

<400> SEQUENCE: 28

Leu Val Ser Val Leu Ser Lys
1 5

<210> SEQ ID NO 29
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 2403, ORF1a: 738, Nsp2: 355,
passage 17 amino acid

<400> SEQUENCE: 29

Leu Asn Ser Leu Leu Ser Lys
1 5

<210> SEQ ID NO 30
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 2403, ORF1a: 738, Nsp2: 355,
passage 52 amino acid

<400> SEQUENCE: 30

Leu Asn Ser Leu Leu Ser Lys
1 5

<210> SEQ ID NO 31
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 3019, ORF1a: 943, Nsp2: 560,
passage 0 amino acid

<400> SEQUENCE: 31

-continued

Ala Pro Met Tyr Gln Asp Glu	
1	5
<210> SEQ ID NO 32	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 3019, ORF1a: 943, Nsp2: 560, passage 17 amino acid	
<400> SEQUENCE: 32	
Ala Pro Met Cys Gln Asp Glu	
1	5
<210> SEQ ID NO 33	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 3019, ORF1a: 943, Nsp2: 560, passage 52 amino acid	
<400> SEQUENCE: 33	
Ala Pro Met Cys Gln Asp Glu	
1	5
<210> SEQ ID NO 34	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 4477, ORF1a: 1429, Nsp3: 72, passage 0 amino acid	
<400> SEQUENCE: 34	
Cys Ala Pro Lys Gly Met Asp	
1	5
<210> SEQ ID NO 35	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 4477, ORF1a: 1429, Nsp3: 72, passage 17 amino acid	
<400> SEQUENCE: 35	
Cys Ala Pro Thr Gly Met Asp	
1	5
<210> SEQ ID NO 36	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 4477, ORF1a: 1429, Nsp3: 72, passage 52 amino acid	
<400> SEQUENCE: 36	
Cys Ala Pro Thr Gly Met Asp	
1	5
<210> SEQ ID NO 37	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	

-continued

<220> FEATURE:
<223> OTHER INFORMATION: genome position 4705, ORF1a: 1505, Nsp3: 148,
passage 0 amino acid

<400> SEQUENCE: 37

Pro Lys Val Val Lys Val Ser
1 5

<210> SEQ ID NO 38
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 4705, ORF1a: 1505, Nsp3: 148,
passage 17 amino acid

<400> SEQUENCE: 38

Pro Lys Val Ala Lys Val Ser
1 5

<210> SEQ ID NO 39
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 4705, ORF1a: 1505, Nsp3: 148,
passage 52 amino acid

<400> SEQUENCE: 39

Pro Lys Val Ala Lys Val Ser
1 5

<210> SEQ ID NO 40
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 5985, ORF1a: 1932, Nsp4: 129,
passage 0 amino acid

<400> SEQUENCE: 40

Ala Gly Glu Leu Val Gly Val
1 5

<210> SEQ ID NO 41
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 5985, ORF1a: 1932, Nsp4: 129,
passage 17 amino acid

<400> SEQUENCE: 41

Ala Gly Glu Ile Val Gly Val
1 5

<210> SEQ ID NO 42
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 5985, ORF1a: 1932, Nsp4: 129,
passage 52 amino acid

<400> SEQUENCE: 42

Ala Gly Glu Ile Val Gly Val
1 5

-continued

<210> SEQ ID NO 43
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 7419, ORF1a: 2410, Nsp7: 217,
passage 0 amino acid

<400> SEQUENCE: 43

Ala Asp Phe Asp Pro Glu Lys
1 5

<210> SEQ ID NO 44
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 7419, ORF1a: 2410, Nsp7: 217,
passage 17 amino acid

<400> SEQUENCE: 44

Ala Asp Phe Asn Pro Glu Lys
1 5

<210> SEQ ID NO 45
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 7419, ORF1a: 2410, Nsp7: 217,
passage 52 amino acid

<400> SEQUENCE: 45

Ala Asp Phe Asn Pro Glu Lys
1 5

<210> SEQ ID NO 46
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 8831, ORF1a/lb: 2881, Nsp9:
429, passage 0 amino acid

<400> SEQUENCE: 46

Gln Thr Pro Val Leu Gly Arg
1 5

<210> SEQ ID NO 47
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 8831, ORF1a/lb: 2881, Nsp9:
429, passage 17 amino acid

<400> SEQUENCE: 47

Gln Thr Pro Ile Leu Gly Arg
1 5

<210> SEQ ID NO 48
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: genome position 8831, ORF1a/lb: 2881, Nsp9:
429, passage 52 amino acid

-continued

<400> SEQUENCE: 48	
Gln Thr Pro Ile Leu Gly Arg	
1 5	
<210> SEQ ID NO 49	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 13,857, ORF5: 29, passage 0	
amino acid	
<400> SEQUENCE: 49	
Ala Val Leu Val Asn Ala Asn	
1 5	
<210> SEQ ID NO 50	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 13,857, ORF5: 29, passage 17	
amino acid	
<400> SEQUENCE: 50	
Ala Val Leu Gly Asn Ala Asn	
1 5	
<210> SEQ ID NO 51	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 13,857, ORF5: 29, passage 52	
amino acid	
<400> SEQUENCE: 51	
Ala Val Leu Gly Asn Ala Asn	
1 5	
<210> SEQ ID NO 52	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 14,578, ORF6: 74, passage 0	
amino acid	
<400> SEQUENCE: 52	
Val Ala Leu Thr Met Gly Ala	
1 5	
<210> SEQ ID NO 53	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 14,578, ORF6: 74, passage 17	
amino acid	
<400> SEQUENCE: 53	
Val Ala Leu Ala Met Gly Ala	
1 5	
<210> SEQ ID NO 54	
<211> LENGTH: 7	
<212> TYPE: PRT	

-continued

<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 14,578, ORF6: 74, passage 52	
amino acid	
<400> SEQUENCE: 54	
Val Ala Leu Ala Met Gly Ala	
1 5	
<210> SEQ ID NO 55	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 14,780, ORF6: 141, passage 0	
amino acid	
<400> SEQUENCE: 55	
Pro Gly Ser Thr Thr Val Asn	
1 5	
<210> SEQ ID NO 56	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 14,780, ORF6: 141, passage 17	
amino acid	
<400> SEQUENCE: 56	
Pro Gly Ser Ile Thr Val Asn	
1 5	
<210> SEQ ID NO 57	
<211> LENGTH: 7	
<212> TYPE: PRT	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: genome position 14,780, ORF6: 141, passage 52	
amino acid	
<400> SEQUENCE: 57	
Pro Gly Ser Ile Thr Val Asn	
1 5	
<210> SEQ ID NO 58	
<211> LENGTH: 24	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: oligonucleotide primer	
<400> SEQUENCE: 58	
gcagagctcg ttaattaaac cgtc	24
<210> SEQ ID NO 59	
<211> LENGTH: 83	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: oligonucleotide primer	
<400> SEQUENCE: 59	
aaaaaaaaa aaaaaaaaaa atgcatatTT aaatcccaag ccgaattcca gcacactggc	60
ggccgttact agtgagcggc cgc	83

145

The invention claimed is:

1. A vaccine for protecting a porcine animal against infection by a PRRS virus, which vaccine comprises (a) a polynucleotide molecule of SEQ ID NO: 6 or a polynucleotide molecule that has at least 85% identity to SEQ ID NO: 6, wherein the protein encoded by ORF1a thereof has an amino acid sequence that contains:

(1) one, two, three, or four of:

an amino acid N within the amino acid sequence ANV (see SEQ ID NO: 9);

an amino acid N within the amino acid sequence HNA (see SEQ ID NO: 12);

an amino acid Y within the amino acid sequence WYL (see SEQ ID NO: 18); and

an amino acid H within the amino acid sequence VHG (see SEQ ID NO: 21); and

(2) one or both of

an amino acid T within the amino acid sequence ATD (see SEQ ID NO: 27); and

an amino acid C within the amino acid sequence MCQ (see SEQ ID NO: 33); and

(3) one or both of

an amino acid T within the amino acid sequence PTG (see SEQ ID NO: 36); and

an amino acid A within the amino acid sequence VAK (see SEQ ID NO: 39); and

(4) an amino acid N within the amino acid sequence FNP (see SEQ ID NO: 45); or

146

(b) a North American PRRS virus encoded by said polynucleotide molecule of (a) or,

(c) said polynucleotide molecule in the form of a plasmid, or

(d) a viral vector comprising said polynucleotide molecule, wherein the PRRS virus is able to elicit an effective immunoprotective response against infection by PRRS virus, in an amount effective to produce immunoprotection against infection, and a carrier suitable for veterinary use, and wherein said vaccine provides early and safe vaccination as early as when the piglet is 1 day of age, and wherein said vaccine provides a duration of immunity for up to 6 months.

2. The vaccine of claim 1 wherein onset of immunity is provided beginning at two weeks after vaccination.

3. A method for vaccinating a porcine animal against infection by a PRRS virus, comprising administering the vaccine of claim 1 to said porcine, wherein said porcine is between about 12 hours after birth and 2 weeks of age and wherein the duration of protective immunity provided is up to 6 months.

4. The method of claim 3 wherein protective immunity arises no later than about 14 days after vaccination.

5. The method of claim 4, wherein protective immunity arises at Day 15 following vaccination on Day 1 of life, Day 21 following vaccination on Day 7 of life, or no later than about Day 28 following vaccination on Day 14 of life.

* * * * *